A Note About the Cover

The cover illustration is drawn from a field photograph of the Langur or Black-Faced monkey, Presbytis entellus. It was from the body of one of these monkeys that Kyasanur Forest disease virus, the study of which has occupied the major portion of the efforts of the Virus Research Centre during 1958, was first isolated. By their deaths these animals have focused attention on the presence of KFD virus, and it is therefore appropriate that one should grace our cover.

[1] The Virus Research Centre is jointly maintained by the Indian Council of Medical Research and the Rockefeller Foundation. It is located in premises provided by Bombay State.

2 This report is privately circulated and its appearance is not considered to constitute publication. Results of original work reported herein should not therefore be referred to in print without the permission in writing of the Director of the Virus Research Centre.

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1. INTRODUCTION

The Virus Research Centre is primarily concerned with the investigation of ARthropod-BORne (ARBOR) viruses which at some point in their life history infect man. As these agents for the most part have as hosts a great variety of both wild and domestic mammals and birds (and possibly reptiles as well) many disciplines must be represented on the team which seeks to unravel the story of how they are maintained in nature. Apart from the actual investigation of these agents, the VRC is concerned with the training of a nucleus group of Indian scientists to develop the outlook and the skills which will enable them to effectively attack problems in this field.

The overall initial design had been that of serologically surveying samples of the human population to develop some concept of the distribution and incidence in India of already known arthropod-borne human infections and to seek out as yet unknown agents. This work has progressed to the point where it is now evident that a substantial portion of persons in India, by the time they reach adulthood, have experienced some ARBOR virus infection. The magnitude of the general problem is thus already established.

Attention has been diverted from this broad aim by epidemic outbreaks of diseases with an ARBOR virus etiology. There has thus been a shifting in emphasis to the working out of the epidemiology of these agents which are the cause of serious human illnesses. Concentration of effort is now centered about the problem of Japanese B encephalitis and West Nile infections in South India and Kyasanur Forest disease in Western Mysore State. As time can be taken from these pressing problems which are both scientifically interesting and of public health importance, further surveys in ecologically diverse regions of India are being continued. The present report outlines the work which has been carried on in both directions.

A report of this sort could be constructed in a number of ways. We have chosen to utilize a structure which emphasizes the epidemiological orientation of the work as it is carried on at the VRC. The laboratory is considered primarily as a working base in support of the field epidemiological investigations. Field epidemiology is stressed as it is through this approach that information of the greatest immediate value to the nation can be gotten while at the same time contributions can be made to a basic understanding of the way virus agents are maintained in nature - a matter of interest and importance throughout the tropic and temperate regions of the world.

Information relating to each particular field problem is thus for the most part gathered together and dealt with in the field epidemiology section. Residual problems are reviewed under the headings of the laboratory sections where the work has been done although investigations are often of an inter-sectional nature and it is therefore necessary to be arbitrary about where a particular bit of work is presented.

2. FIELD EPIDEMIOLOGICAL STUDIES

2-1 Kyasanur Forest disease, Shimoga District, Mysore State.

Kyasanur Forest disease is an arthropod-borne virus infection discovered in Sorab and Sagar taluks of Shimoga District, Mysore State, in 1957. The earliest report of the occurrence of the disease has been traced to a retrospectively diagnosed case at the village of Kannur at the end of December 1955 and to reports of monkey deaths early in December 1955. As evidenced by monkey deaths and human cases the disease continued to be active during the dry season months of 1956, subsided later in the year and again appeared during the dry season of 1957. With the advent of the monsoon in 1957 human cases were no longer seen but monkey deaths in the epizootic area continued. The same general picture has been observed during 1958.

The etiological agent is a virus closely related to that of Russian Spring Summer encephalitis, diphasic meningo-encephalitis of eastern Europe and European Russia, Omsk haemorrhagic fever of western Siberia, louping ill of Great Britain and TP 21, a virus isolated from ticks off rodents in Malaya.

During 1958 the major portion of the activities of the VRC was directed at elucidating various aspects of the natural history of the Kyasanur Forest disease virus through studies of human infections and the mammal, bird and arthropod fauna of the field area together with experimental work in the laboratory.

The understanding of the epidemiology of the disease is constantly undergoing revision as new information is acquired but we may summarize the present state of our knowledge as follows.

1. It still cannot be said with certainty whether or not the virus was indigenous to the area now involved prior to 1955 but the evidence thus far accumulated is consistent with the interpretation that the agent is new in the epizootic area. If this is true there is to be faced the problem of how it arrived where it is. For the present this must remain a matter for speculation.

2. The virus has been repeatedly isolated from ticks and not from other concurrently collected haematophagus arthropods and it is therefore presumed that ticks are the vectors. Virus has most frequently been isolated from <u>Haemaphysalis spinigera</u> and it has been shown in the laboratory that this species is capable of transmitting the virus transtadially and infecting clean animals by bite.

3. The period of maximum incidence of human cases and monkey deaths falls in the time of year when immature stages of <u>Haemaphysalis</u> ticks are at maximum abundance. This is the time from January or February until June, the dry season period.

4. Human cases occur in villagers living in close proximity to the forest and virtually all infections are consistent with a history of exposure in the forests where <u>Haemaphysalis</u> ticks are abundant. Histories of tick bite have, however, been difficult to obtain and to support the thesis that ~~humans contract~~ the infection by tick bite intensi[illegible] remains to be done.

when examination of all sera is completed.

One of the seven deaths was definitely attributed to KFD. This was in a 30-year old male who died on the eighth day of illness. He had leucopenia and passed tarry stools at least once. KFD virus was isolated from blood serum on the fourth day of illness and from blood serum and CSF on the seventh day.

The role of KFD infection in the death of a second case is not clear. The patient, a 25-year old male, was admitted with a history of fever for 15 days. He had leucopenia at the time of admission. He died two days later on the seventeenth day of illness. KFD virus was isolated from the serum specimen on the fifteenth day of illness but not from the seventeenth day specimen collected post mortem. Specimens of skeletal muscle, lung, liver, spleen, kidney and brain collected in glycerine and tested three days later were all negative. The post mortem record notes "congestion of posterior wall of stomach (?) haemorrhage and consolidation of the right lung with purulent fluid in the right pleural cavity."

In the other five illnesses which ended in death, acute blood specimens were obtained on days four, four, five, eight and eight of illness and were negative. In one of them the final diagnosis of "?haemorrhagic smallpox or chicken pox" was considered and in another the "exposure" in infected area was while driving a bus. As the virological results are negative and there are no firm clinical or epidemiological grounds for diagnosis of KFD for these illnesses, the five deaths are not considered as due to KFD virus. In confirmed cases of KFD, virus isolation attempts in the first eight days of illness have seldom been unsuccessful.

Table 1 gives the breakdown of all cases studied, by time of onset. The figures in the last row indicate that the search for cases has been continued through the year. The large majority of proved cases have occurred from February through May with single infections in January, June and December.

Table 1? gives the age and sex distribution of the laboratory proved cases. The male:female ratio is 7:1 and no cases have occurred in persons under the age of ten.

Positive cases in 1957 and/or 1958 have been recorded from 26 villages with a total population of 3,957. Nine villages were found infected in both years, 11 only in 1957 and six only in 1958. The extent of detected clinical human infection in 1958 is therefore 15 villages with a population of 2,605 (1951 census). The 47 positive cases were from a total of 133 examined in these 15 villages. Study of 134 cases from an additional 59 villages did not yield a single positive case. From the 11 villages which were positive in 1957 and negative in 1958, 18 cases were examined. Fourteen were negative and in four the diagnosis was undetermined. If some of the sera under examination now prove positive, the extent of detected infection in 1958 would be increased by two or three villages.

It is of interest to review the data available for the analysis of evidence of spread of the human infection. Two of the six localities viz. Kanahalli and Bhadrapur are both within the area of 1957 infection and

were included in the survey of human sera in 1957. KFD positive sera were found in both places, though no cases were detected that year. The other four localities were not surveyed in 1957. Two of them viz. K. Kappalgadde and Kumbatti are within the area of 1957 infection. In 1958, therefore, human infections in "new" places without a previous history of involvement were detected in two villages viz. Padavagodu and Maduba Siddapur, each contributing a single case. Padavagodu is contiguous to and Maduba Siddapur less than two miles from the periphery of the area of 1957 infection. In 1957 no febrile illnesses from these villages were investigated for the diagnosis of KFD. In 1958, 16 cases were reported of which 11 were examined satisfactorily, with diagnosis positive in two and negative in nine instances.

It would thus appear that in terms of recognized human infection there has not been a marked spread of the disease in 1958.

2-1-2 The vaccine

Arrangements had been made in 1957 for the production of approximat 50,000 doses of vaccine for use in the protection of residents of the Kyasanur Forest disease area. The vaccine was produced by the Biologica Products Research Laboratory in the Division of Immunology of the Walter Reed Army Institute of Research, Washington D.C. the expense being met out of a special KFD grant made by the Rockefeller Foundation.

In January vaccination of the residents of villages in which KFD cases had occurred and a ring of villages peripheral to these was begun by the Mysore Department of Public Health. The vaccine used was a formalin inactivated RSSE strain in mouse brain. The dose schedule called for three inoculations of 1 cc. each; the first two a week apart and the third five weeks later. By the end of 1958 22,394 doses of vaccine had been administered, 2,168 persons receiving one inoculation, 3336 two inoculations and 4518 three inoculations. The vaccinations were carried on in approximately 90 villages. It is anticipated that all villages from which proven cases have been reported and the villages peripheral to these will have received inoculations by February 1959.

For study of antibody response to the vaccine a group of government employees was vaccinated and blood samples taken before each inoculation and following the last inoculation.

2-1-2-1 Serologic response to the RSSE vaccine as measured with KFD virus in haemagglutination inhibition, complement fixation and neutralization tests.

The serologic response to the RSSE vaccine has been studied with KFD virus in HI, CF and Neutralization tests. In all instances paired sera (pre and post vaccination) have been examined and both members of the pair have been included in the same test. A substantial proportion of the vaccinees in this study (Mysore State Government employees) had Group B HI antibodies in the pre-vaccination specimens and so it has been possible to examine whether the previous Group B infection has improved the response to vaccine. What these other Group B infections are, is not known with certainty at the present time, but the CF and HI patterns are suggestive of

dengue infection.

(1) HI Tests: The results of the HI tests are divided into (a) those with Group B HI antibodies in the pre-vaccination serum and (b) those without Group B HI antibodies in the pre-vaccination serum and are summarised in Table 2.

The HI response is not marked in either group, but seems somewhat greater in the first group. In the first group 47.5% of the pairs do not show any rise as compared to 89% in the second; greater than four-fold rise (three tubes and more) is observed in 7.8% of the first group and in 3.3% of the second.

(2) CF Tests: As shown in Table 3, no rise in CF antibodies was detected in the 66 persons with Group B CF antibodies in the pre-vaccination serum or in the 331 persons whose pre-vaccination sera were negative for Group B CF antibodies.

(3) Neutralization Tests: The neutralization tests were done intraperitoneally in two-day-old mice. The source of virus was lightly centrifuged 10% mouse brain suspension stored at -50°C. The sera were collected eight-12 months before testing and were stored at -20°C.

In all instances, the interpretations are based on comparison of results of both members of the pair from an individual. The term 'partial protection' is used when the post-vaccination serum does not protect all mice but gives an Average Survival Time (AST) significantly* greater than the AST with pre-vaccination sample. In 13 pairs, pre-vaccination sera were found partially protective and their results are excluded from the summary.

Table 4 gives the summary of results of 77 pairs with negative pre-vaccination sera. In run # 590, the test virus dose was 3.2 log LD_{50}. All 27 pre-vaccination sera were clearly negative and not one of the post-vaccination sera converted to clearly protective. In six post vaccination sera, significant prolongation of the AST (partial protection) occurred.

In runs # 592, # 593 and # 595, the test virus dose was between 0.7 and 1.0 log LD_{50}. Of the 50 pairs in these tests, 21 post-vaccination sera were clearly protective and 16 more gave partial protection.

In Table 5, the results are analysed to see if the vaccine response differed when the pre-vaccination sera contained Group B HI antibodies. It is evident that the presence of Group B antibodies did not improve the response.

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* When greater by more than three standard deviations. Standard deviation derived for each test from ASTs of all negative -vaccination sera.

In summary, the RSSE vaccine evokes a very limited HI antibody response and no CF antibody response against KFD antigens. In intra-peritoneal neutralization tests in infant mice, sensitive tests with low virus doses detect some response in the majority of vaccinees, but with higher test doses the proportion of vaccinees reacting becomes considerably reduced. Thus, with a virus dose of 1.0 log LD_{50} or less, 42% of the pairs showed full protection and 74% full or partial protection. With 3.2 log LD_{50} virus in the test, none demonstrated full protection and 22% gave partial protection.

The major drawback of this study is that the vaccine response has not been measured simultaneously against the homologous RSSE virus and so no standard is available with which to compare the response to KFD. In any case, the knowledge of the serologic response to this vaccine does not enable one to predict the degree to which it will be effective in the prevention or modification of disease. The effectiveness of a vaccine ca only be measured by a direct test in the field.

2-1-2-2 The problem of the field evaluation of the effectiveness of the RSSE vaccine

The following analysis is based on data available through November 1

Vaccinations with RSSE vaccine have been carried out in 89 villages having a total population of 15,747 according to 1951 census figures. On the criterion that one or more laboratory proved human cases in a village indicates infection of the village, this population can be divided as follows:

	Number	Population
Villages infected	27	4,191
Villages not known to be infected	62	11,556
	89	15,747

A total of 8,169 persons of the population (52%) had received one or more doses of the vaccine; 3177 (19.5%) a full course of three doses, 3,271 (21%) two doses and 1,771 (10.5%) only one dose.

Since the second and third doses are still being administered in certain localities, the above percentages do not represent the expected situation before the epidemic season in February. The following breakdown made on the basis of 14 villages with a population of 2,143 in which the vaccination programmed has been concluded, may be taken as representin picture when the vaccination programme is completed.

No. of Villages	Population	Total Vaccinated	1 dose only	2 doses only	3 doses only
14	2143	1186	211	411	564
Percentage	100	55	10	19	26

Eleven lots of vaccine have been administered over a period of 11 months. The recommended schedule of doses at zero, seven and 35 days has not been possible for all cases and wide variations have occurred.

The evaluation of the vaccine rests on the comparison of attack rates and clinical severity of the cases in the vaccinated group with that in a 'comparable control group'.

The lack of homogeneity of the vaccinated group: The vaccinated group is not homogenous in many respects, viz.

(1) Less than half the persons who originally volunteered for vacc tion took a complete course. The proportion of those taking 3, 2 and 1 doses is roughly 47:36:17. One does not know if the distribution in thr groups is random or is brought about by some definite factors such as age, sex, occupation and place of residence, which are important in the epidemi logy of KFD.

(2) Variations in the schedule of inoculation have occurred. No information is available how this would affect the pattern of immunity.

(3) Time between the last vaccine dose and the epidemic season will vary from 2-3 weeks to as much as 12 months in the vaccinees. This variable is of considerable importance if the immunity is not long lasting.

(4) Several vaccine lots have been used at different times after their preparation. Little information is available on the stability of the vaccines. This, however, can be obtained.

(5) The same proportion of the population is not vaccinated in each village. Persons having one or more doses of vaccine constitute from 20 to 90% of the population in different villages. Since the KFD infection is so spotty, this lack of homogeneity introduces a variable of considerable importance.

(6) The sex and age distribution of the vaccinees is not uniform for all villages. Table 17 gives an analysis of 1126 vaccinees from 14 villages. The male:female ratio in the vaccinees as a whole is 59:41 but varies between 47:53 and 67:33 in the individual villages. Since the sex-specific risks differ greatly, the shifting male:female ratio can bias the results of vaccine effectiveness. Likewise, ratios between various age groups show considerable variation.

Unvaccinated controls: The exact number of "unvaccinated controls" is not known and can only be roughly guessed by subtracting the number vaccinated from the 1951 census figures. Attack rates cannot be computed for the unvaccinated without an accurate knowledge of their number.

The unvaccinated consist of:

(1) Those above five years old who repeatedly refused vaccination or were not present at the time of vaccination.

(2) Children under five years.

(3) Ninety persons, mainly adults, shown to have antibodies to KFD in HI tests in 1957 survey and so regarded as not needing vaccination. Some of these, however, have antibodies due to infection with group B agents other than KFD and the capacity of these to prevent or modify KFD infection is not known.

It is evident that analysis of vaccine effectiveness cannot be done for every village and it will be necessary to pool data from many villages in an area. Since the exposure to KFD varies from locality to locality and the age and sex specific risks are markedly different, the poolin the data will introduce a bias of unknown magnitude.

In the unvaccinated group the children under five and the 90 per with 'antibodies' have to be obviously excluded since there are no comparable groups in the vaccinated. The number and age and sex distr bution of the remainder will have to be ascertained.

Even if adjustments are made for the difference in the sex ratios and the age distribution is found broadly comparable in the unvaccinated and the vaccinated, it is questionable if the unvaccinated can be considered a 'control group'.

The unvaccinated above five years are those who either did not care to take the vaccine or actually refused it, and in that respect are different from those who volunteered for vaccination. It is possible that the vaccinees may come largely from a higher socio-economic-cultural group and may for that very reason, have lesser risks of infection. On the other hand, they might report more promptly in the event of an infection and may show a greater willingness to permit taking specimens resulting in a greater efficiency in diagnosis in the vaccinated. Do the vaccinees have the same exposure as the unvaccinated? Will the vaccinee, knowing that he has been immunized, take greater risks of forest exposure?

The magnitude of the bias introduced by these factors or even its direction is not only unknown but unknowable. To this will be added the unconscious bias of the physician resulting from his knowledge about the vaccination status of the patient and his own feeling about the vaccine.

In a disease like KFD, with its spotty distribution, marked differences in age and sex specific risks, and with its association with forest exposure well known to the inhabitants in the endemic area, a proper evaluation of a specific vaccine does not seem feasible in the absence of a placebo controlled trial. Randomization of the volunteers into vaccinees and placebo controls after ensuring equal distribution in the two groups with regard to sex, age, locality and occupation, lack of knowledge on the part of the physician and the volunteer as to in which...

group any individual volunteer belongs, and objective criteria of diagnosis; these would be necessary for an unbiased evaluation of the vaccine effectiveness.

However, a study of the disease in the two groups as they are now can be made while avoiding unwarranted conclusions. The following suggestions may provide a basis for discussion on how the study may be done most fruitfully.

Table 18 gives an age and sex distribution of the laboratory proved cases of 1957 and 1958. Eighty-eight per cent of the cases (83/95) are in males over ten years of age.

Table 19 gives the age and sex distribution of 1126 vaccinees of 14 villages. In this group about 17% of the total vaccinees are under ten years of age. Males over ten years of age constitute about 50% of the vaccinees. Twenty-six per cent of these or 13% of the total vaccinated would be males about ten who have had three doses of vaccine.

For a preliminary analysis, the following is proposed.

(1) To consider only the male of greater than ten years of age for both the vaccinated and unvaccinated groups.

(2) To examine the age distribution of the male above ten in both groups and if widely dissimilar, to make corrections for these based on age specific attack rates in final computations.

(3) To assume that the vaccinated are a random selection of the males above ten as far as risk of exposure is concerned.

(4) To consider in the study only those villages where one or more laboratory proved cases in males over ten have occurred and then assume that these localities have comparable infection risks.

(5) To compare attack rates in unvaccinated with those vaccinated with three doses. Variations in vaccine schedules should be studied.

(6) To compare attack rates in those receiving one, two and three doses.

One must accept that each of the assumptions mentioned above is potentially hazardous and at least some of them may have to be revised.

Will there be enough cases? Increase in the population at risk by finding of cases in villages heretofore believed unaffected, and/or increase in the attack rate would result in a greater number of cases and would improve the chances of getting an indication of vaccine effectiveness. Since it is desired to spot every possible case of KFD, practically all cases of fevers should be subjected to laboratory scrutiny. It is now common experience that though the classical case of KFD may be unmistakable clinically, some cases may elude clinical diagnosis. Again, the vaccine may modify the course of illness and an over suspicious attitude with a minimum of clinical discrimination is likely to be the most productive.

An estimate of the attack rate in the general population may be made from the figures in Table 20. It is interesting to note that fewer villages and a smaller population were found affected in 1958 as compared to 1957. While nine villages were affected both times, 11 were affected only in 1957 and six only in 1958. In the infected villages (villages with cases that year) the attack rate for the general population may be guessed very roughly as a little more than two per hundred and for the males over ten at five-six per hundred.

With trepidation, one can speculate on the number of cases to be expected in the vaccinated males over ten years who have received three doses of vaccine if the attack rate was 5/100 and the vaccine totally ineffective (Table 21). This is done for three possibilities: (1) if proved cases occurred in each village found infected in 1956, 1957 or 1958; (2) if proved cases occurred in each of the 89 vaccinated villages; (3) if cases occur in an area similar to that of 1958. (These calculations are made as a mental exercise and not as predictions).

The essence of the scheme presented is (1) to concentrate on the male over ten years since the children and females contribute few cases, and introduce errors by diluting the vaccinated and the unvaccinated groups to different degrees; (2) to neglect all villages where cases in males over ten have not occurred. This will limit the population to be studied and so permit more effort for an accurate determination of the number and characteristics of the two groups, more especially the unvaccinated group. As the study progresses it will doubtless be essential to modify these ideas but the purpose of presenting them at this time is to provide a basis for discussion which, one hopes, will lead to clarity of thought and improvement in the study. All computations made are approximate, and at various places extrapolations are made from results of examination of small samples.

2-1-3 Monkey mortality during 1958

Monkey deaths are a prominent feature of Kyasanur Forest disease. These are the most abundant of the conspicuous wild mammals of the KFD area. Two species are present, the black-faced or langur monkey (Presbytis entellus) and the red-faced or bonnet macaque (Macaca radiata). It was the report of these monkeys dying which first drew the attention of the VRC to the Kyasanur Forest disease problem, and it was from a langur monkey which died in Kyasanur Forest that the virus was first recovered in 1957.

At the end of 1957 monkeys had died in an area of 618 square miles. On December 31st 1958, the area in which monkeys died was enlarged to 771 square miles. A summary of the monthly distribution of reported monkey deaths during 1958 is given in Table 6. In all there were 117 monkey deaths reported of which 98 were identified as Presbytis entellus and four as Macaca radiata, the remainder being unidentified as to species. During August, at the height of the monsoon, only a single monkey death was recorded. This is the time when tick activity is at a minimum, but also a time when people are not moving about in the forest and the chance of coming across dead monkeys is correspondingly reduced.

Of the 117 monkey deaths reported, 21 bodies were reached in time to obtain post-mortem tissue specimens for possible virus isolation. Virus was isolated from six of these 21 specimens. The isolations were all from monkeys which had died in the proximity of villages from which there have been proven human cases of Kyasanur Forest disease. The area in which there have been monkey deaths proven by virus isolation to be attributable to KFD is thus very much smaller than the total area over which monkeys have died. Proven KFD monkey deaths have occurred in an area of 77 square miles by comparison with the 771 square mile figure for all monkey deaths. All virus isolations were from Presbytis entellus, but this species comprised 19 of the 21 monkeys autopsied.

During the coming year it is planned to introduce a reward system for reports of dead monkeys to improve the chances of getting earlier autopsy specimens and enhance the possibility of virus recovery, particularly in those areas outside the known infected area.

2-1-4 Small mammal surveys during 1958

The interpretation of the results of neutralization tests with KFD virus on sera obtained from wild-caught rodents can be made at the present time only on a provisional basis. As soon as possible specimens of the various species will be tested for susceptibility to KFD virus in the laboratory and data obtained which will allow more definite interpretations to be made.

At the present time only a few Rattus rattus wroughtoni and Rattus blanfordi have actually been inoculated with KFD virus and have had their sera tested in neutralization test. It is known that each of the species is susceptible to KFD virus and that they actually develop neutralizing antibody. As seven Rattus rattus wroughtoni have been examined and none were found to have sera containing non-specific viricidal substances it would seem that the virus neutralizing activity of the field sera was probably due to the presence of antibodies. As only two specimens of Rattus blanfordi have been tested there exists less certainty about the significance of a virus neutralizing serum. Both species though were found to circulate the virus. In one species (RRW) some animals which had circulated virus developed only sufficient neutralizing antibody to produce what is labelled a "partially protective" (PP) result in the accompanying table (Table 8). Therefore, it would seem most likely that the "partially protective" results observed in the field caught rodents also indicated past infection with KFD or a closely related virus.

The results obtained with the squirrel sera were the most encouraging, especially noting that most of the sera are fully protective which in most circumstances has been found to be the situation in a highly susceptible species. Unfortunately there are no laboratory data to help interpret the results with this species. A closely related species, Funambulus pennanti, has however been found to be very susceptible to KFD virus. So many of the experimental animals died, apparently of the infection, that only a single convalescent serum has been available for study. This serum contained large amounts of virus neutralizing antibodies. No information is yet available on virus circulation and antibody development in the shrew and the forest mouse. Thus at the present time the virus neutralizing

activity in the serum of these animals suggests the desirability of testing these animals in the laboratory. The laboratory work done thus far is reported in Section 3-1-4.

During the year small mammal trapping was carried on for one week of each month at three localities where there was evidence of KFD virus activity in 1957. These places were Kyasanur Forest adjacent to the village of Barage and the forests near the villages of Hosur and Kannur. The results of the neutralization tests on 454 small mammal sera tested are shown in Table 8. The mammals in which positives were found were *Rattus rattus wroughtoni*, *Rattus blanfordi*, *Funambulus tristriatus*, *Mus booduga* and *Suncus murinus*. Each of these species gave clear positives as well as "partial protective" results. There were no species which gave "partial protective" results without at least one positive result. The animal with the highest incidence of positives was the palm squirrel, *F. tristriatus* of which 4/22 (18 per cent) were positive and an additional 2/22 (nine per cent) "partially protective". The commonest small mammal, *R. r. wroughtoni*, proved disappointing as an indicator of virus activity, as only a single animal of 278 tested gave a clear positive (0.4 per cent) and six others (two per cent) were "partially protective". It would appear from the preliminary laboratory experiments done (see Section 3-4-1) that these rats have a minimal response to KFD virus infection and the low incidence of wild-caught animals for which NT antibody can be demonstrated may not be a valid measure of those which, in fact, may have been bitten by infected ticks. From the fact that of seven *R. r. wroughtoni* which gave some protection in NT only one was a clear positive, while four of the six *F. tristriatus* which reacted were clear positives, there is confirmation of the laboratory infection experiments indicating that *F. tristriatus* is a better host for the virus.

Apart from the routine monthly collection of rodents at the three localities discussed above two other sorts of small mammal collections were made.

For one, localities were selected which fringed the KFD epidemic area to determine if small mammals could be used as a more sensitive measure of virus activity than is human infection. The plan was to trap at each locality until a sample of approximately 30 small mammals had been obtained but in some places where the catches were low, the trapping was discontinued after fewer animals had been gotten. The data from localities at which no positives were found are summarised in Table 7 and those with some positives in Table 8. The technique has proved in some measure a success as positives or "partial protectives" have been found in the forests adjacent to villages in which there is as yet no overt sign of KFD in the human population, although these are villages in which survey collections of human sera have either not been made or not as yet run. Localities from which there is some evidence of KFD in small mammals but not yet in humans are Amanchi, Gamataghata, Hasivi, Kolisal and Kuppe. It has, however, been somewhat disappointing to not find antibodies in small mammal samples from forests adjacent to villages where human cases have occurred as for example, Dugur, Jimmoor and Kodakani.

The second group of "spot" collections were made as part of a survey of the tick fauna along lines radiating in various directions from the known KFD area for approximately 100 miles. Small mammals were collected primarily to determine their tick ectoparasite load for comparison with that found in the KFD area, but the animals were bled as well and their sera run in neutralization tests. The data on the negative animals are summarised in Table 7. A surprising and even startling result has been the finding of an NT positive in one of two field mice, Mus booduga, taken at Arehalli (see Table 8). No laboratory experiments have as yet been done to determine the susceptibility of this species to KFD virus infection or to check on the specificity of the neutralizing substances in its blood, but it is noteworthy that one of 12 specimens of this species taken near Barage in Kyasanur Forest was also positive. Arehalli is in Hassan district about 100 miles from the known KFD area. The significance of this finding will be explored in the coming year.

2-1-5 Serological survey of cattle sera

From the results of serological examination of rodent and human populations in the KFD area, these appear not to be ideally sensitive indicators of virus activity. Even in places known to be infected, fair sized survey samples of rodents or humans have few or no serological positives. In the KFD area, the livestock has presumably much greater exposure to the virus than do humans as the suspected vector ticks are found on them in substantial numbers and these animals graze widely in the forest. The livestock population (including domestic fowl) is larger than the human population in the area, and cattle and buffaloes are the commonest domestic animals. If cattle and buffaloes are susceptible to KFD virus infection and developed specific antibodies they might prove to be sensitive indicators of virus activity. A small preliminary survey has been made of sera of animals from infected and uninfected areas.

Sera were collected from 21 bullocks, three goats, three cows, four buffaloes and two heifers from the village of Kuppagaddi in the non-infected area, and from 23 bullocks, 12 cows and eight buffaloes from the villages of Shigga, Hosur and Hunavalli in the centre of the infected area.

In intracerebral neutralization tests in mice, all the 33 sera from the uninfected area were negative while 13 out of the 43 (30%) from the infected area were positive. The positives were 5/23 bullocks, 4/12 cows and 4/8 buffaloes.

All sera were negative in complement fixation tests and all but one in HI tests. The one HI positive serum was from an NT positive animal. It gave a low but reproducible titre of 1:10 to KFD and was negative to other group B antigens. The complementary study of experimental infection of a buffalo calf is reported in Section 3-4-4-5 of the "Laboratory studies".

These results along with the data on viraemia and serological response in the transmission study, indicate that the neutralization of the virus is specific, and that survey of cattle sera by neutralization test may provide a useful tool in mapping out areas of infection.

thirds of the 387 Haemaphysalis nymphs were H. spinigera. Of the 66 Haemaphysalis adults, ten were H. minuta and 56 H. wellingtoni. As many as 17 H. wellingtoni adults were collected from a single jungle fowl. The only Ixodes ticks on birds in the area was a single nymph collected from a jungle fowl in Koppalagadde.

The infestation rate of small mammals with ticks in Kyasanur, Kannur and Hosur Forests is shown in Table 14. Out of a total of 673 animals trapped and examined during the 12-month period October 1957-September 1958, roughly half (49 per cent) were infested with ticks. The infestation rates for each species are given in Table 15. About one-tenth of the number of forest mice, Mus booduga, examined were infested with ticks. The highest infestation rate, 71.9 per cent, was seen in the palm squirrel, Funambulus tristriatus.

Out of a total of 3,005 ticks of all stages collected from small mammals, 2,376 were Ixodes (1,955 larvae and 413 nymphs and eight adults of I. ceylonensis); 284 ticks were Haemaphysalis and about three times as many nymphs as larvae of this genus were collected. The number of Dermacentor ticks collected was roughly the same and in this case also more nymphs than larvae were represented in the collections. The rest of the ticks in the collection were composed of larvae, nymphs and a few adults of Rhipicephalus and a single nymph of Amblyomma.

The numbers of Haemaphysalis and Ixodes larvae, nymphs and adults collected per infested small mammal during the different months are given in Table 16. Ixodes larvae and nymphs were collected during all the months, the maximum number of larvae being found in May and the maximum number of nymphs in August. Adults of this genus were collected only in May, July and August. The number of Haemaphysalis larvae collected per animal was 1.5 in January and this may be correlated with the first peak of Haemaphysalis larval activity in November-December (Fig. 1). During or following the second peak of larval activity in February and March, there did not appear to be any significant increase in the number of Haemaphysalis larvae collected per small mammal. More Haemaphysalis nymphs than larvae were collected from small mammals. A little less than half (100 out of 213) of these nymphs were H. spinigera and the majority of this species was taken from Rattus blanfordi and Funambulus tristriatus.

On cattle and buffaloes, larvae, nymphs and adults of Boophilus microplus were the commonest ectoparasites. Adults of Haemaphysalis spinigera were also collected from these animals in fair numbers. Larvae and nymphs of Haemaphysalis, adults of H. formosensis, H. papuana, H. turturis, H. bispinosa, H. cornigera, Rhipicephalus sanguineus and R. haemaphysaloides, nymphs of Amblyomma and adults of Amblyomma integrum and A. testudinarium were also represented in the cattle and buffalo ectoparasite collections.

2-1-6-3 Survey collections

During February, March and April 1958, a number of survey tick collections were made in localities outside the epidemic area in Shimoga District and also in the neighbouring districts of Hassan, Chikmagalur, Chitaldrug, South Kanara and North Kanara. The collections were from

ground drags as well as ectoparasitic collections from trapped rodents and insectivores and cattle. The purpose of the survey collections was to compare the tick fauna of these areas with that of the epidemic area. The terrain where the collections were made varied from open pasture land through scrub jungle, bamboo forest and deciduous forest to thick, broad-leaved evergreen forest. The rainfall in the areas sampled varied from low to high (up to 350 inches per year) and the collections were made from places up to 4000 ft. in altitude. Thus a fairly representative collection of ticks was obtained from a variety of ecological situations. The collections have not been completely identified yet and since they were made during a period when only immature stages were mostly active, it is difficult to enumerate all the species represented. Haemaphysalis larvae and nymphs (at least three species of nymphs including H. spinigera) were common. Haemaphysalis papuana and H. cuspidata adults were collected from ground drags in deciduous, semi-deciduous and evergreen forests in N. Kanara district and H. spinigera adults were collected from ground drags in a bamboo forest in Sringeri (Chikmagalur District) and also from bullocks in Karwar (North Kanara District). Haemaphysalis parva adults which have not so far been recorded from the epidemic area in Shimoga district were taken many times in ground drags and as cattle ectoparasites in the districts of Chitaldrug, Hassan, Chikmagalur, South Kanara and North Kanara. Hyalomma larvae which have not been collected in Shimoga district were recovered from ground drags in Tarikere, Chikmagalur district and Kumaranhalli and Kunduwada in Chitaldrug district. On the other hand Dermacentor larvae, nymphs and adults which were collected in the epidemic area, although in much smaller numbers than Haemaphysalis, were totally unrepresented in the survey collections. Ixodes nymphs were taken twice in Chikmagalur district, once from Suncus murinus and once from Rattus rattus wroughtoni. The commonest cattle ectoparasite in the districts of Chikmagalur, Hassan, Chitaldrug and N. Kanara, where cattle were examined, was Boophilus microplus, followed by H. parva and H. bispinosa.

In addition to the species already mentioned, the following ticks were represented in the survey collections: H. turturis adults, Rhipicephalus larvae and nymphs, adults of Rhip. haemaphysaloides, Boophilus larvae and nymphs, Hyalomma sp. adults and Amblyomma larvae and nymphs.

2-1-6-4 Ticks dropping off cattle in the forest and in cattle sheds.

For this study, four villages were taken, namely Horabail, Bhadrapura, Dugur and Ulvi. At the beginning of each run, all the ticks were collected from three to five marked cows or bullocks leaving usually ten adult ticks (the number varied from two to 18) which were then marked and numbered. The cattle were thereafter examined twice every day, in the morning before they were taken out into the forest for grazing and in the evening when they were brought back to the cattle sheds in the villages after grazing. A record was kept of the number of marked ticks which were still attached to the animals at each of these examinations. It was presumed that ticks which were on the animal in the morning and absent in the evening had dropped off in the forest and those which were on the animals in the evening and absent in the morning, had dropped off in the cattle sheds. The observations were continued for each animal until all the numbered ticks had dropped off. The results varied a great

deal, more ticks presumably dropping off in the forest from some animals than from others and vice versa. Out of a total of 189 ticks followed in Horabail from June to September, 101 (53.4 per cent) presumably dropped off in the forest and 88 in the cattle sheds. The figures for Bhadrapura were 84 (51.2 per cent) in the forests and 80 in the cattle sheds. In Dugur it was 73 ticks (68.2 per cent) in the forests and 34 in the cattle sheds and in Ulvi 67 ticks (58.3 per cent) in the forests and 48 ticks in the cattle sheds. However, since the marked ticks were not identified as it was not feasible to do this in situ on the animals, there was no way of knowing which species actually dropped off in the forests and which in the cattle sheds, a point of considerable epidemiological importance. The majority of ticks collected from the marked cattle in all the four villages in June, when the studies were started, was Boophilus microplus. Haemaphysalis spinigera was also represented in the collections although in much smaller numbers.

2-1-6-5 Ticks which infest cattle while grazing in the forest and in cattle sheds

This study was conducted from June 1958 onwards in the same four villages of Horabail, Bhadrapura, Dugur and Ulvi where the experiments with marked ticks were done. The two studies were done simultaneously. At the start of each run, five heads of cattle were selected and numbered and all the ticks on them were collected. Thereafter, every day the cattle were examined in the morning before they were taken out into the forests for grazing and in the evening when they were brought back to the cattle sheds. At each examination, all the ticks found attached to the cattle were removed. It was presumed that ticks which were taken attached to the cattle when they came back in the evening had attached in the forest, since all the ticks on the animals had been removed in the morning and ticks which were attached to the cattle in the morning had attached whilst the animals remained in the cattle sheds overnight. The majority of ticks collected from cattle in this study consisted of nymphs and adults of Boophilus microplus and adults of Haemaphysalis spinigera. Since both these species were recovered in the morning and evening collections, it has to be presumed that they attached to the cattle in the cattle sheds as well as in the forests. However, unless one is certain that all the ticks attached on the cattle, including those which can be clearly seen and those which are hidden by the hairs are removed at each examination, one doubts whether the ticks found attached to the animals in the morning were already there but missed in the examination the previous evening.

2-1-6-6 Collection of ticks from cattle sheds

From June onwards, cattle sheds in a number of villages from which the animals are taken out regularly into the forest for grazing were searched for ticks. In some villages, no ticks could be recovered from the cattle sheds whilst from cattle sheds in other villages, a few and in some cases several hundred ticks were collected. In Horabail for example, two fed Boophilus microplus were obtained from cattle sheds on two different occasions. The finding of the fed females in a particular place is important since it gives evidence that the species is potentially capable of breeding there, provided conditions in the cattle sheds are such that the female can survive and lay eggs. Nymphs, males and fed females of

H. bispinosa were taken in small numbers from cattle sheds in three other villages. In Kuppe village, a two man-hours search yielded over 1600 ticks which were collected from cracks and crevices in the mud walls. The adults were male and female H. bispinosa, fed and unfed. The majority of the nymphs, also H. bispinosa, were unfed as were the larvae. Besides these, several hundred tick eggs were collected and many of them hatched out in Poona into Haemaphysalis larvae, probably H. bispinosa. Thus there was definite evidence in this case that breeding was taking place in the cattle sheds. No other species of ticks were collected from the cattle sheds although cattle in these villages are usually infested with H. spinigera.

2-1-7 Other arthropods

In addition to ticks, collections have been made routinely of other haematophagus arthropods of the KFD epizootic area. These include mosquitoes taken attacking man, day and night, on the ground and in the tree canopy of Kyasanur Forest and also resting on vegetation during the day in several forest localities where there has been other evidence of KFD virus activity. Mosquitoes gotten in these ways have been inoculat into mice at the Vellore field station. During the year there were no isolations of KFD virus from this material. The 24 hour collections on the ground and in the forest canopy which had been begun in June 1957 were discontinued at the end of May 1958 as there was no evidence suggesting the possible involvement of mosquitoes in the KFD virus cycle. The data from these collections when analysed will provide the first information on the biting activity cycles of forest mosquitoes in India, information which will doubtless prove of value in the understanding of other ARBOR virus infections as time progresses. It is planned to work up these data during the coming year.

During the first half of the year collections of Phlebotomus sand-flies resting in the forest were made in continuation of the general collecting program begun in 1957. The making of these collections was stopped in June as no evidence of Phlebotomus involvement in KFD transmission had been obtained.

Mite material has not been inoculated for virus recovery but the mites taken attached to small mammals and monkeys have been preserved and stored for later systematic study.

2-2 Japanese B encephalitis and West Nile studies at Vellore, Madras State

2-2-1 Human cases of JBE. (Contributed by Dr. John Webb)

In October, November and December 1958, further cases of Japanese B encephalitis were seen at the Christian Medical College, Vellore. Twelve of the 14 cases clinically consistent with JBE and for which there is supporting virological or serological evidence have been in children aged 14 years or less, but this year the diagnosis has been confirmed in adults also. After four years of investigation, virus isolations from human cases have at last been made. These are the first isolations from human material in India. Of the 14 cases, nine appear to have made good

recoveries, one has severe residua, and four have died. Although permission for complete autopsy was not given in any of these four cases, brain tissue was obtained immediately after death from three of them, once with a Vim-Silverman biopsy needle passed through the still patent anterior-fontanelle, and twice through burr-holes. The brain tissue obtained was inoculated intracerebrally in infant mice and virus was recovered in each instance. These children had died on the fifth, seventh and ninth days of illness. In one other case, a boy aged thirteen years under the care of Dr. Jacob Chandy, brain biopsy through a posterior burr-hole was performed on the third day of illness: from this tissue also virus was isolated. This child went on to make an apparently complete recovery.

2-2-2 Entomological Studies

During the year a gradual shift in emphasis of the entomological aspects of the field program at Vellore was made with the introduction of bullock baited stable traps. A series of these traps were made and installed at villages where JBE cases had been seen in previous years near Vellore. Collection of mosquitoes by this means, it is felt, will more accurately reflect relative mosquito population densities than the hand-catching of resting mosquitoes, the method being replaced for the long-term abundance cycle studies. The hand-catching method continues to be of value in making spot collections for getting large numbers of mosquitoes for possible virus isolation when this is indicated. In conjunction with the installation of one group of stable traps, maps have been prepared of the surrounding agricultural land, the individual plots given code numbers, and the stage of the water and crop cycle recorded twice a month for each plot. It is hoped that this will give information which will be helpful in correlating mosquito densities with local agricultural practices.

It has been found that what has been called Culex "vishnui" is actually a mixture of several species of closely related mosquitoes. These can be separated as larvae but as yet no reliable characters for the identification of adult females have been found. Pressure of other work has not permitted the concentrated study by senior personnel required to resolve this problem. A beginning towards sorting out the main features of the bionomics of the species involved has been made, however, by concentrating studies on the readily identifiable larvae.

2-3 Japanese B Encephalitis, Chintamani, Mysore State.

In December, on learning from the Assistant Surgeon, Chintamani Hospital, that four cases of encephalitis in children had been seen during the preceding several weeks, this place was visited and blood samples obtained from the three surviving patients. They were bled on the 19th 29th and 31st days of illness. The CF titres of these sera indicated possible recent JBE infection. This isolated finding viewed in conjunction with the concurrent cases of JBE seen at the Christian Medical College Hospital, Vellore, suggest that JBE virus was again active in a widespread area of south-east India during late 1958. As the facilities of the VRC were so fully occupied with work on the KFD problem it was not possible to devote time to following up this interesting finding.

2-4 Entomological investigations in the Poona area, Bombay State

2-4-1 Arthropod collections from Loni and Manjri

Studies by D.H. Colless on Singapore Island have shown that *Culex "vishnui"*, is actually a complex of several closely related species. To study the composition of the "vishnui" population around Poona and also to obtain specimens for the continued efforts to colonise *C. "vishnui"*, mosquitoes were collected from the environs of Poona using a stable trap. In December 1958, the trap with bullock bait was set up in a farm in Loni, 11/5 miles east of Poona on the Poona-Sholapur road. In January 1958, the trap was shifted to a farm in Manjri 7/5 miles E of Poona on the same road, and was run till the first week of March 1958 with a buffalo calf as the bait. Engorged specimens of *Culex "vishnui"*, *C. fatigans*, *C. bitaeniorhynchus*, *C. fuscocephalus*, *Anopheles stephensi*, *A. splendidus*, *A. theobaldi*, *A. fluviatilis*, *A. subpictus*, *A. hyrcanus*, *A. tessellatus*, *A. annularis*, *A. turkhudi*, *A. moghulensis* (?), *A. culicifacies*, *Phlebotomus argentipes* and *Culicoides* sp. were collected.

Several engorged larvae and nymphs of *Haemaphysalis bispinosa* as well as many fed *Hyalomma* larvae were collected from the outside and inside walls and door joints of the stable trap in Loni where the bullock had been exposed overnight. A few of the engorged ticks were found as high as seven feet on the inside walls of the trap. Many of the *Haemaphysalis* nymphs were found to be parasitized by chalcid wasps.

2-4-2 Entomological investigations at the National Defence Academy, Khadakvasla, Poona District, Bombay State.

Following the outbreak of an epidemic disease among the riding horses of the National Defence Academy, mosquito collections were made from the horse stables as part of the epidemiological investigations. A total of 36 collections involving 60 man hours were made from March 9 to April 19 and 1289 female mosquitoes were collected. Large numbers of male *Culex* and *Anopheles* also were collected which indicated the proximity of breeding place *Culex fatigans* was the predominant mosquito, followed by *Anopheles subpictus* and an unidentified species of *Culex*. A total of 154 mosquito blood meal smears were sent to Dr. B. Weitz of the Lister Institute of Preventive Medicine, London, for determination of the source of blood meals. Out of the 131 *C. fatigans* tested, 117 were found to have fed on birds, four on man and three on horses. In contrast to this, out of 22 blood meals from *Anopheles subpictus*, *A. culicifacies*, *A. splendidus*, *A. turkhudi* and *A. fluviatilis* tested, 20 were positive for horses.

2-5 Survey of Saurashtra, Bombay State

2-5-1 Serological survey of six localities

In the 1952 survey for antibodies to ARBOR viruses in sera of adult residents of India, Smithburn, Kerr and Gatne detected neutralizing antibodies to RSSE virus in eight out of 588 sera. Six of the eight came from a group of 26 adults from Kutiyana, Saurashtra and this led the authors to conclude that a virus of the RSSE group had been active in that region in recent years.

After the discovery that a newly recognised illness, Kyasanur Forest disease in Mysore, is caused by a virus of the RSSE group, it was decided to seek more information about the incidence of anti-bodies to this group of viruses in Saurashtra. Two-hundred-and-forty human sera were collected for this purpose from the six localities shown in Table 22.

Kutiyana proved to be a compact walled town surrounded by dry, open groundnut and cotton fields. For the other bleedings the attempt was made to sample populations in small villages or hamlets in close proximity to forests. The entire area is however one of low rainfall, and forest, where it could be found, was open and deciduous. Bhanvad, Killeshwar and Khambala were three localities in the Barda Hills inland from the port town of Porbandar. Daulatpura was a settlement at the edge of Junagadh with a community living in close association with an extensive second growth plantation of teak forest. The Gir Forest collections were among people living in hamlets consisting of only a few houses surrounded by the forest which is a game preserve maintained for the preservation of the less than 300 remaining Asiatic lions. These people graze water buffalo in the low, open deciduous forest and have the most intimate forest contact of any of the groups bled in Saurashtra.

The sera were examined for HI and CF antibodies against the following antigens: Sindbis (AR 339) of group A; Tr 1751 strain of dengue II, JBE, Egypt 101, G 2266 strain of WN and KFD of group B. None of the sera reacted with the Sindbis antigen. A high proportion showed HI antibodies against the group B antigens. The highest titres in both HI and CF tests were generally but not always against the dengue antigen, and the HI and CF pattern appeared consistent with dengue infection. In Table 22, a summary of the results is given in terms of HI and CF antibodies to group B viruses.

In Bhanvad, Kutiyana and Daulatpura, the incidence of group B HI antibodies was as high as 83-100% in the age group above 15, and 44-59% in the younger group. In the other three localities of Khambala, Gir Forest and Killeshwar, the incidence in both groups was lower. Males had, on the whole, a somewhat higher incidence than the females. The ratio between HI positive / CF positive varied between 2:1 to 4:1 in the different localities indicating that at least some of the infections were recent.

In intracerebral neutralization tests against KFD virus, 24 out of 240 sera (10%) were found positive (Table 23). All the 24 positives were from individuals of 15 years of age or above and the four youngest donors with positive sera were of 15, 15, 16 and 17 years of age.

For age group 15 and above, the overall incidence of positives was 14%; 18% for the females and 12% for the males.

Each of the six localities had one or more persons having neutralizing antibodies to KFD. In five of the localities the incidence of antibodies in adults ranges from three-14% while in Kutiyana 41% of the adults (14/34) had neutralizing antibodies.

Table 24 lists the HI and CF titres of the 24 donors positive to KFD in neutralization test. In spite of the fact that these sera are positive for KFD in neutralization tests, three out of 24 show no antibodies to KFD in HI test and two react only in the dilution of 1:10. (In proved cases

possible to make more definite interpretations after completion of neutralization tests of these sera.

3. LABORATORY STUDIES

3-1 Animal virology section

3-1-1 New isolations

The agents isolated from human, animal and tick sources, which have proved to be strains of Kyasanur Forest disease (KFD) virus, have been discussed in the epidemiological section. In addition, a number of other agents have been isolated.

Twenty-five strains of virus have been isolated from ticks of various species and stages caught in the Sagar area. The pertinent information now available on these strains has been summarized in Table 28. It will be noted that several of the strains were isolated from ticks collected as ectoparasites from various animals and birds. Frequently, the larval stage was found to contain virus. In most instances in which an attempt has been made to reisolate the virus, the attempt failed.

Two viruses, virulent only for infant mice, were isolated from mice inoculated with serum from two human cases from the Sagar area. (Table 29.)

Table 30 summarizes the information on the mosquito isolates from the North Arcot District of Mysore State. Several of these viruses belong to group B, but none have been completely identified.

Four viruses have been isolated from children suffering from encephalitis in the Christian Medical College at Vellore, North Arcot District. Three of the patients died and virus was isolated from post-mortem brain specimens. The fourth virus was isolated from a brain biopsy sample and the patient has recovered from the acute illness. Two of these viruses have been found to be quite closely related to the Japanese B encephalitis virus. The third strain was not tested by the end of the year and the fourth strain had not been received from the field laboratory as yet. (Table 31.)

During the early part of the year an epidemic of encephalitis occurred in horses maintained at the National Defence Academy at Khadakvasla, just outside Poona. Viruses were isolated from the serum of one horse and from the kidney of another. The two viruses behaved similarly in mice. It was not possible though to prove that these isolates had any etiological significance when they were tested with the sera obtained from horses surviving the disease. A dog, which was found sick in the vicinity of the stables, was shown to be suffering from rabies. (Table 32).

A virus was also isolated from a case of encephalitis from Vengurla, Bombay State which is situated on the west coast of India just north of Goa. The history of this patient is somewhat complicated and all of the details are not entirely certain. It appeared that the patient went to Vengurla to be at her home when her child was born, which event occurred on August 13, 1958. The child survived for only eight days. On the

11th post-natal day the patient had a convulsion and was unconscious for about 24 hours. On the 15th post-natal day her face became swollen, a condition which lasted for two days and then slowly subsided. During this time it was noticed her urine was scanty in amount. Following the convulsion she developed rigidity of the upper extremities and stiffness of the neck which was present when she was admitted to the hospital on September 9, 1958. During her stay in the hospital and at the time a blood sample was drawn the weakness of the arms and neck stiffness had only been somewhat lessened. The blood sample was drawn on September 23rd and was received in Poona on September 27th, 1958, uniced. (As far as we have been able to tell, the blood was probably held two days on ice in Vengurla before it was sent to Poona). The patient had diplopia for far vision, some difficulty in swallowing and an ataxic gait. From this blood sample a virus was isolated which was shown by complement-fixation to be closely related to West Nile virus. A convalescent sample was obtained from the patient on November 22, 1958 and when tested with the virus isolated previously, and with the "acute" sample, it was found that both samples neutralized the virus. An attempt to re-isolate the virus was successful to the extent that an agent with similar characteristics in mice was obtained. The re-isolation has not been confirmed serologically as yet.

3-1-2 Laboratory infections with KFD

During 1958 there have been eight proven laboratory infections with KFD virus. Seven of the infections occurred in persons previously inoculated with RSSE vaccine. Interestingly enough, these have occurred in two outbreaks of four cases each. The grouping of the cases would suggest some incident leading to infection but thus far such an incident has not been found.

3-1-3 Comparison of the effectiveness of KFD and RSSE vaccines in monkeys inoculated with KFD virus.

Each group of ~~twelve~~ three monkeys was inoculated subcutaneously with one of the vaccines, KFD or RSSE, after having been bled to obtain a control sample. The second dose of vaccine was given seven days after, and the third dose 21 days after the first dose. One-hundred-and-one days after the first dose of vaccine the monkeys were challenged, a pre-inoculation blood sample having been obtained from each monkey. Experiments had revealed that the intravenous route of inoculation was the route of choice and that a relatively large dose was necessary to kill most of the animals, something approximating 100,000 LD_{50} for mice. Daily post-inoculation bleedings were titrated in mice for as long as indicated. Post-infection samples for antibody studies were also collected where indicated. The results of this experiment have been summarized in Figures 2 and 3. Figure 2 presents the combined graph of the four control monkeys. Figure 3 presents the comparison of viremia of control monkeys as compared with the RSSE and KFD vaccinated monkeys.

It was very interesting to note that R176 monkey gave no evidence of having an infection in that no circulating virus was detected nor were detectable antibodies produced. It was considered quite certain that the monkey was inoculated intravenously. One explanation proffered was that the virus was exactly neutralized thus combining with all of the available

presence of circulating virus. Seven R. r. wroughtoni and two R. blanfordi were tested. (See Table 33). The rats were not very large and it was thought risky to try and bleed each daily. They were consequently bled at staggered intervals to try and cover an eight day possible circulation range which was from the second through the ninth post-inoculation days. Each rat was bled to obtain a pre-inoculation reference sample and again about a month after inoculation to try and determine the development of neutralizing antibodies.

Five of the seven of the R. r. wroughtoni and one of the two R. blanfordi circulated virus. The animals that circulated virus showed some evidence of having developed virus neutralizing antibodies but in one or two instances the activity was minimal. One of the R. r. wroughtoni whi[illegible] did not circulate did develop antibody. On the whole, the amount of vir[illegible] circulated was not great and none of the animals showed overt signs of the infection. The peak titre was 4.0 logs while most titres were in the range of 0.6 -2. logs. The animals circulated virus between the 2nd and 6th post-inoculation days.

3-1-5 Effect of glycerination on virus material

It has been the practice to put material collected in the field in glycerine for storage in the refrigerator or deep freeze until it was ready for inoculation. A considerable quantity of this material has accumulated that has not been possible to inoculate and has filled available storage space. Faced with this situation, and also as a matter of general interest as no information on the subject was available, it was decided to test the survival of Japanese B and Kyasanur Forest disease virus in glycerine in the cold (4°C.) With this type of information in hand it would be possible to determine from what date backward it would be possible to discard material from which it would be highly unlikely to isolate virus, had virus been present in the sample at the outset.

Sixty brains were harvested from groups of mice sick with one of each of the above viruses. At this time three brains infected with each virus were titrated individually in adult mice inoculated intracerebrally. The remainder of the brains were placed individually in tubes containing at least five parts of glycerine, and stored in the refrigerator. The attempt was made to simulate actual procedures as much as possible. At intervals, three brains infected with each virus were removed, washed three times in normal saline, and titrated separately in adult mice inoculated intracerebrally. The log titers thus obtained were arithmetically averaged and the result plotted on a graph. (See Fig. 4).

It appears from the graph that (1) both viruses lose about 90% of their activity during the first few days, (2) thereafter the rate of loss is progressively less and (3) JBE virus is slightly more labile than KFD. The rather rapid loss of virus during the early part of the experiment was somewhat unexpected and suggests that more work should be done in ascertaining the effectiveness of this procedure as a field technique. In this connection two other factors should be considered as especially significant: firstly, the amount of virus in field materials is often small; secondly, the susceptibility of mice for these unadapted strains is also often low. Therefore, any procedure which leads to such a high loss of virus may

completely nullify the efforts made to isolate it.

3-1-6 Mouse colony

The mouse colony is a part of a laboratory which, as long as it functions satisfactorily, does not receive comment. At the present time the epidemic which has been raging, as far as can be judged from the history all through 1958, has seriously interfered with the work of t laboratory. The cause of the epidemic would seem to be a gram-negati bi-polar staining bacillus which produces a disease in mice clinically and pathologically like the mouse disease caused by Pasteurella pseudo-tuberculosis. Strenuous attempts have been made to control this disea but it is not at present certain whether or not it can be eradicated fr the colony.

3-2 Tissue Culture section

3-2-1 Kyasanur Forest disease (KFD) virus

Two strains of this virus isolated from human serum (P 9605) and monkey serum (W 372) have been maintained in monkey kidney (MK) and chick-embryo cells (CE) tissue cultures. The various observations made during the year can be summarised as follows:

(1) Strain W 372 after 75 serial passages in CE tissue culture and strain P 9605 after more than 100 serial passages in MK tissue culture, have not shown any significant changes in their capacity to produce paralysis or in their incubation period when inoculated intracerebrally into mice.

(2) It is possible to do identification tests with P 9605 virus in MK tissue culture on the basis of inhibition of cytopathogenic effects. It is proposed to develop a neutralization test on this line.

(3) It has been possible to prepare CF antigen in HeLa cell cultures with P 9605 virus at times, but this technique does not always give re-producible results.

(4) Plaques with the P 9605 strain of KFD virus in MK tissue culture were obtained. In initial experiments the size of the plaques were larger than those produced by West Nile virus. It is proposed to carry out passages from selected plaques for standardization of plaques.

(5) It has been observed that cytopathogenic effect (CPE) produced by P 9605 virus varies with the medium used. Using MK tissue culture system and three different media, the following observations were made:

(a) M+Hanks medium: There is no complete degeneration of cells even ten-12 days after inoculation. Cells are very granular.

(b) M+Earles medium: Cytopathogenic effects were observed between the second and fifth day after inoculation, depending on virus concentration. Cell degeneration was complete by the tenth to twelfth post-inoculation

(c) BME medium: Cytopathogenic effects were delayed by one or two days as compared with M+Earles medium. Otherwise the observations were the same as with M+Earles medium.

3-2-2 Enteric viruses.

3-2-2-1 Vellore

It is now an established practice to investigate, if necessary, clinical cases of poliomyelitis admitted to the Christian Medical College Hospital, Vellore. This is done for differential diagnosis of arbor virus infections, particularly when the clinical picture is not clear. As in past years, polio type I virus has been isolated from cases. Also one type II virus has been isolated during the year.

3-2-2-2 Outbreak of encephalitis in Nagpur

An outbreak of encephalitis similar to that in Jamshedpur in 1954 occurred in Nagpur in 1958. The Virus Research Centre took part in investigating the epidemic in the later part of the outbreak. Stool, blood and CSF specimens were collected from cases. Blood samples were obtained from contacts and from the general population. Stool specimens were inoculated into MK tissue cultures and infant mice for virus isolati

The virus isolates are a heterogenous group. A total of 33 agents have been isolated from 46 stool specimens. Thirty-one of these cause cytopathogenic changes in MK tissue culture. Out of these, five also cause sickness in mice. Two viruses cause sickness in mice only and no cytopathogenic changes are observed in MK tissue culture. Among the agents there have been identified polio type I, polio type II and Cox-B4 (one each). Six others behave like ECHO viruses. Unfortunately a sufficient number of convalescent blood samples are not available to pin-point any particular virus as the etiological agent. It is not unlikely that in such outbreaks, more than one antigenic type of enteric virus may be involved as the etiological agent.

3-2-2-3 Monkey enteric viruses.

In laboratory investigations of KFD virus, Macaca radiata monkeys are sometimes used. In captivity, stock monkeys suffer from diarrhoea and even die at times. Stool specimens of some of these monkeys were tested for virus in MK tissue culture. Fifteen agents have been isolated, which can be divided into two groups on the basis of cytopathogenic changes produced in monkey kidney cells. They do not cause any sickness in infant mice. It is planned to test material from wild forest inhabiting monkeys gotten at the time of their collection to develop some concept of the variety and incidence of agents present under natural conditions.

3-2-2-4 Technical.

During the year certain technical modifications were adopted which may be mentioned. Since 1955 the method of Rappaport has been used for trypsinization of monkey kidney-cells. Essentially this consists of:

(1) initial incubation of monkey kidney fragments in trypsin for one hour, (2) mixing the kidney fragments by magnetic stirrer in trypsin (at approximately 35-37°C.) for seven minutes and then decanting. This process is repeated till virtually all tissue fragments are digested, which takes about two hours. All the cells are used on the same day.

At present, a modification of Bodian's method of cold trypsinization is used. This consists of incubation at 37°C. for one hour before overnight (16-18 hours) trypsinization at 4°C. The following day cells are sedimented by centrifugation and resuspended in the growth medium. This method which is more convenient has proved as good as, if not better, than the one previously used.

3-3 Serology section

During the greater part of the year, this section was engaged in carrying out survey and diagnostic work on sera collected for epidemiological studies from (1) the KFD area in Shimoga District of Mysore State and (2) from the vicinity of Vellore in Madras State for Japanese B encephalitis and West Nile. Results of this work have been discussed in the section on field epidemiology.

A substantial number of paired sera collected from vaccinated individuals were tested by hemagglutination-inhibition (HI) and complement-fixation (CF) tests in an attempt to evaluate serological response of these individuals to RSSE vaccine. This work is covered separately in the field epidemiological section.

Besides these, a number of sera were received from a variety of places for serological tests with ARBOR viruses. Results of interest are discussed below.

3-3-1 Bangalore sera:

During JBE studies in Vellore, a follow-up on children showed some with post-encephalitis sequelae. From the Deputy Superintendent of the All India Institute of Mental Health, Bangalore, it was learnt that a considerable number of children coming to the mental hospital, have a previous history of encephalitis. A small study was undertaken to determine whether there was a high incidence of group B antibodies in children coming to the mental institute with a history of encephalitis. About 50 such sera have been tested for HI antibodies against various group B antigens, viz., Japanese B encephalitis, Egypt 101 strain of West Nile, Tr 1751 strain of dengue II and Kyasanur Forest disease virus. No significant serological results which can be related to the history of encephalitis have been obtained so far.

3-3-2 Colombo, Ceylon, sera

Twenty pairs of acute and convalescent sera were sent by the Medical Research Group of Colombo for serological testing. All except two (one 14 years and the other 40 years of age) persons were in the age group of 15-30. These were tested for CF and HI antibodies against JBE, Murray Valley-encephalitis virus, E 101, Tr 1751 and Sindbis AR 339. None

showed any antibodies for group A, Sindbis virus. Ten individuals did not show detectable CF or HI antibodies against any of the viruses tested. Six others failed to demonstrate any CF antibodies although HI antibodies were detected in all. Two of these, maintained the same level of Group B HI antibodies in both acute and convalescent sera. Of the other four, two showed a rise in HI antibodies for TR 1751 only while two indicated a slight rise in all Group B antigens tested.

Out of the remaining four, two persons showed a slight rise (one, one tube and the other two tubes) against TR 1751; a significant rise was also observed in their HI titres. Two other persons showed presence of CF antibodies for TR 1751 in both the acute and convalescent specimens. HI antibodies for all Group B (most marked for TR 1751) were obtained with these last mentioned sera.

Excepting the two cases where there was a slight rise in CF antibodies, none of the others point to Tr 1751 as a causal agent for the present infection. However, the general CF and HI pattern indicates previous dengue infection, which is further confirmed by the results of Colombo sera tested last year. Out of 21 pairs examined then, nine showed definite conversion both to dengue I and Tr 1751 strain of dengue II. These results clearly indicate dengue activity in Colombo.

3-3-3 Bangkok, Thailand, sera:

During the recent epidemic of haemorrhagic fever in Bangkok, paired sera from 46 children, mostly ranging from two-ten years of age, (six were between ten-15 years) were received at the VRC. These were tested against JBE, E 101, Tr 1751, dengue I, KFD and Sindbis AR 339 antigens. Hemagglutination inhibiting antibodies were present in 45 and complement-fixing antibodies in 36 acute serum samples. Twenty-nine acute sera were obtained within the first week, ten being collected within the first four days. Twelve 'acute' were taken between first and second week and four were collected after 15 days. Since ten of the positive acute sera were obtained within the first four days, it is reasonable to consider the presence of HI antibodies as evidence of previous group B infection, particularly as the titres were high.

HI titres obtained with all antigens including KFD were very high, ranging from 1:640 to 1:20480. CF results also indicated a broad spectrum ranging from 1:128 to 1:2048 for all antigens including KFD: (not so marked for E 101). This is interesting as even in genuine cases of KFD from South India, such high titres have not been observed. The highest titres in most of these sera from Bangkok, were generally for dengue. Last year, 70 sera from children of similar age group showed presence of HI antibodies for dengue in 75% of the specimens tested. The pattern is definitely that of a secondary Group B infection involving dengue and it appears that there is more than one virus active in the area.

This picture is further confused by some eight-nine pairs showing a fall, both in CF and HI titres of convalescent sera. Perhaps this may be due to an anamnestic reaction which reaches its peak in early convalescence, gradually falling thereafter. In one instance, (six days acute),

high CF titres against JBE, E 101 and KFD began to fall in 18 days although dengue titres remained high. The same specimen showed a fall in HI titre for KFD only. However, this does not hold good in most instances, where a fall occurs for all antigens including dengue. At least in one instance an 11 days 'acute' sample showed CF titres ranging from 64-256 which disappeared completely for all antigens by the 26th day. High HI titres also dropped to insignificant levels. Results of this sort have not been previously observed and suggest the possibility that there may have been some confusion of serum pairs.

Fourteen sera show a rise in CF titres for all antigens and 18 for HI. Because of this great overlap in CF and HI titres and a variation in the collection days of acute samples, it is very difficult to interpre these results.

Some of the sera showing high CF titres for KFD were studied in neutralization test with this virus. The more sensitive intraperitone test for baby mice was used and both survival ratios and the ASTs were compared. None of the sera gave protection against KFD. Tests with JBE and dengue viruses are yet to be completed.

3-3-4 Stockholm, Sweden, sera:

Thirty-nine human sera were received from Dr. A. Svedmyr of the Central Bacteriological Laboratory of Stockholm, for serological tests with KFD virus. These included twelve pairs of acute and convalescent sera which showed conversion to HYPR (a Czechoslovakian strain similar to RSSE) by CF test and fifteen others which were negative for this agent.

CF and HI tests were done using Sindbis AR 339 of group A and KFD, JBE, E 101 and Tr 1751 of group B. Sera which were negative to HYPR strain were also found completely negative both by CF and HI to all the antigens tested. The 12 pairs which showed conversion with HYPR antigen also converted for KFD although the CF titres with the latter antigen were always one or two tubes lower. This may be due either to the fact that HYPR is antigenically more closely related to the RSSE-like agent active in Stockholm than is KFD, or to difference in technique. The latter possibility could not be completely eliminated, as at present, no work with members of RSSE complex other than KFD is being done at the VRC. None of the sera showed CF antibodies for any of the other viruses tested.

Every one of the 21 (three acute samples were exhausted) sera tested for HI showed presence of these antibodies against KFD, there being invariably a rise in convalescent sample. Four sera showed antibodies to JBE, E 101 and Tr 1751 and three for JBE and E 101 only. However, in all cases, these were at a very low level, one or two tubes as compared to four or five tubes or more for KFD.

Theiler and Casals have observed a distinction in antibody patterns between "primary" and "secondary" infection with group B agents. Results obtained with the Stockholm sera tested would seem to support this as the cold climate population concerned might be expected to be relatively free

of group B infections and the RSSE group infections are "primary" ones which in the HI test give a clear specific reaction. It is interesting to note here that a similar clear HI pattern of 'primary infection' has also been observed in some cases of KFD (with no previous group B antibodies) in South India.

3-3-5 General

The large volume of complement-fixation and hemagglutination-inhibition tests performed in support of the field program and preparation of all the necessary reagents, together with cramped working quarters, limited other activities of this section. However, efforts were continuously made to modify techniques in order to increase the efficiency of these tests.

Work is continuing with some encouraging results and it is hoped to perform all the routine HA and HI tests on plastic plates using goose erythrocytes. At present, we are also working on preparation of non-infectious antigens by use of beta-propiolactone (BPL), an agent known to inactivate bacteria and viruses. Following the methods reported in literature, successful CF antigens have been obtained though some difficulties are encountered in preparing high titred and stable HA antigens. Both control and BPL treated antigens show comparable titres soon after treatment with BPL but on storage at -50°C the HA activity seems to deteriorate in a very marked manner in four to seven days. This work is yet in a preliminary stage and attempts will be continued to get over this initial difficulty.

3-4 Experimental Entomology section

3-4-1 Insectary

The stock colonies of Culex fatigans, Aedes albopictus and Anopheles stephensi var. mysorensis were maintained without difficulty.

3-4-2 Breeding of Culex "vishnui"

Attempts to breed the species in a 6'x6'x8' high outdoor cage, with females (most of them gravid) from Vellore, Akividu and Kanjri, were unsuccessful.

3-4-3 Rearing of ticks

The following species of ixodid ticks are being colonised in the laboratory: Haemaphysalis spinigera, H. bispinosa, H. parva, Amblyomma integrum and Hyalomma sp.

For associating the immature stages of ixodid ticks with the readily identifiable adults, unfed immature ticks collected from Shimoga district are reared to the adult stage and engorged live ticks collected as ectoparasites are held for moulting. It is now possible to distinguish larvae and nymphs of Haemaphysalis spinigera and nymphs of H. bispinosa, H. papuana, H. wellingtoni, H. turturis, H. cuspidata and H. formosensis. However a more extensive series of associated stages will be necessary before a key for distinguishing the immature stages of the different

species can be prepared.

3-4-4 Infection of mosquitoes and ticks with the virus of Kyasanur Forest disease

Infection studies were done with three different strains of the virus, W 372 isolated from the blood of a monkey, P 9605 from human serum and G 11338 isolated from a pool of *Haemaphysalis* larvae and nymphs.

3-4-4-1 Infection of mosquitoes with W 372 by inoculation

Culex fatigans, *Aedes albopictus* and *Anopheles stephensi* var. *mysorensis* were inoculated with $10^{-1.3}$ dilution of third passage mouse brain suspension having a titre ≥ 8.5 in baby mice. In *fatigans*, ther was detectable virus after one day, five days and 20 days, but none on t tenth, 15th, 25th, 30th and 35th days after inoculation. Attempts were made to pass the virus serially in *fatigans*, but without success. In *A. albopictus*, the virus after inoculation was detected upto 45 days, whe the last surviving mosquitoes were tested. Attempts to pass the virus serially in this species also were unsuccessful. In the *Anopheles* mosqui toes, the virus was detectable up to 15 days after inoculation when the last pool of mosquitoes was tested. Inoculated mosquitoes of all the three species were fed on baby mice but there was no evidence of transmission.

3-4-4-2 Infection of mosquitoes with P 9605 by inoculation

Culex fatigans and *Aedes albopictus* were inoculated with 10^{-1} dilution of second passage mouse brain suspension having an adult mouse ic titre of 8.6. In *fatigans*, there was detectable virus after one, six, ten and 25 days, but none on the 15th, 20th and 30th days or thereafter till the 45th day. In *albopictus*, virus was present for up to 25 days after inoculation. Second passage mosquitoes of both species were negative for virus content when tested 16 days after inoculation. Mosquitoes of both species were fed on one-day old chicks, but there was no evidence of transmission.

In a second experiment, mosquitoes were inoculated with $10^{-0.7}$ dilution of third passage mouse brain suspension having an adult mouse ic titre of 9.4. Virus was detectable till the 20th day in *fatigans* and 25th day in *albopictus* when the last surviving mosquitoes were tested. A test-feed of *albopictus* on a 2-3 day old chick after 19 days incubation was negative.

3-4-4-3 Infection of mosquitoes with G 11333 by feeding

Three two-three day old chicks and three five-week old hamsters were inoculated subcutaneously with third passage infected mouse brain suspensi Starting from the first post-infection day, batches of *C. fatigans*, *A. albopictus* and *Anopheles stephensi* var. *mysorensis* were fed on these animals. All the donor animals circulated virus. The fed mosquitoes were tested at five-day intervals by triturating pools of five and inoculating the suspensions into adult mice. In the case of *C. fatigans* none of the eight pools tested upto 25 days after the infecting feed had any detectable virus, nor was there any evidence of transmission of the virus to clean chicks by bite. None of the 46 pools of *A. albopictus* tested upto 55 days after the infect-

ing feed contained detectable virus and there was no evidence of transmission to chicks by the bite of the mosquitoes. Similar results were obtained with the Anopheles mosquitoes and none of the five batches tested upto 25 days contained detectable virus.

In a second experiment, one-two day old chicks were inoculated subcutaneously with third passage mouse brain suspension. Batches of A. albopictus were fed on two chicks on each of the first three post-infection days. Pools of the fed mosquitoes were tested for virus immediately after feeding and at eight-hourly intervals for the first 24 hours and thereafter every day till the tenth day, then on the 15th day, 20th day and so on.

The two batches of mosquitoes fed on chicks on the first PI day did not pick up any virus and all pools from these batches starting from zero days incubation, were consistently negative. Of mice which were inoculated with the undiluted sera from the donor chicks, one out of four and two out of four only died.

In the two batches which had infecting blood meal titres of 2.3, there was detectable virus in the mosquitoes for up to 16 hours after the feed, not later. In the fifth batch of mosquitoes also, which had an infecting blood meal titre of 3.6, there was detectable virus up to 16 hours after the feed. In the last batch which had an infecting blood meal titre of 4.6, virus was present for at least up to one day, after which no more mosquitoes were available for testing.

3-4-4-4 Infection of Aedes albopictus with P 9605 by feeding

This experiment was done in collaboration with the Virology section. The donor animal was a rhesus monkey which had been inoculated ip with infected third passage mouse brain suspension. On the second, third, fourth and fifth post-infection days, batches of A. albopictus were fed on the monkey. The monkey was bled immediately after feeding was over and the serum titrated by the Virology section. The circulating virus titres were five logs on the second PI day, 6.5 logs on the third PI day, 6.6 logs on the fourth PI day and 3.33 logs on the fifth PI day. Pools of five mosquitoes from the batches fed on the four days were triturated and inoculated into mice immediately after feeding, eight, 16 and 24 hours after feeding, thereafter every day till the fifth day and then on the tenth, 15th, 20th and 25th days. Virus was detectable for up to two days after feeding when the titre of the infecting blood meal was 6.5 and 6.6. With infecting blood meal titres of 5.0 and 3.33 logs, virus was detected only up to one day after feeding.

Sixteen days after the infecting meal, 22 mosquitoes fed on the third, fourth and fifth PI days were allowed to bite and feed on six two-day old chicks. There was no evidence of transmission.

The results from this experiment and the one already outlined using chicks as the donor animals are summarised in Table 34. It may be seen that the virus is detectable in the mosquitoes only up to two days after feeding. During this period the time up to which the virus persists in the mosquitoes would appear to depend on the amount of virus ingested by

the mosquitoes which in turn depends on the circulating virus titre of the donor animals. Since an interval of four-five days usually elapses before the mosquito is ready for the next blood meal, these laboratory experiments would indicate that A. albopictus is probably of no importance in the biological transmission of the virus in nature.

3-4-4-5 Infection of the tick Haemaphysalis spinigera with G 11338 by feeding

The virus was taken through two chick-tick cycles by feeding. Haemaphysalis spinigera larvae were released on four donor chicks about one-and-half days after the chicks had been inoculated with infected blood. Blood drawn from the chicks just before releasing the ticks was titrated in adult mice after 115 days frozen storage at -50oC. Two of the chicks had titres of 1.5 and 2.0 and in the other two chicks there was detectable virus at 10^{-1} (the lowest dilution inoculated), but mortality end-points were not obtained.

Fed larvae dropping off from each chick on successive days were tubed separately. Larvae in the moulting phase and nymphs emerging therefrom, were tested in pools of three to six for the presence of virus by inoculation of the suspensions into mice. Trans-stadial transmission of the virus was shown to have occurred in all the four batches of ticks. Tick suspensions were also turned over to the Tissue Culture section for attempts to isolate the virus in tissue culture systems and also for titrations. Suspensions of unfed nymphs made after 30 days incubation and titrated after 121-128 days storage at -50oC, had titres of 5.3 to 6.8.

Nymphs from all the four infected batches were released on one-eight-day old chicks 24-73 days after the infecting feed and 11 to 60 days after emergence. Transmission was obtained as early as 24 days after the infecting feed and 11 days after the emergence of nymphs and as late as 73 days after the infecting feed and 60 days after emergence. Partly fed nymphs which were either forcibly detached from sick or dead chicks or which detached of their own accord, were released on clean chicks after varying number of days. They fed readily to repletion and transmitted the infection by bite. Out of a total of 41 chicks on which the nymphs attached and/or fed, eight died one to three days after releasing the nymphs and were discarded without passing the brains. Definite evidence of transmission (circulating virus, demonstration of specific HI antibodies in the sera and the presence of KFD virus in bacteria-free suspensions of the brain) was obtained in 25 out of the remaining 33 chicks. In eight chicks there was no evidence of transmission.

Adults emerging from the fed nymphs were tested for the presence of virus by inoculation of the suspensions into mice. The infection rate in the four batches of infected ticks varied from 25 to 70 per cent, with an average of 52 per cent. Both males and females were infected. Virus was detectable in the adults as late as 176 days after the infecting feed as larvae. The titre of the virus in adult ticks varied from 3.8 to 6.0, when the tick suspensions were titrated after 64 to 108 days frozen storage at -50oC.

Infected females were released on two 8-10 months old rabbits. The ticks which fed on both rabbits were shown to be infected by subsequent crushing and inoculation of the suspensions into mice. Both rabbits remained healthy and blood drawn from the rabbits 28 to 47 days after the release of ticks did not have any HI antibodies to KFD virus.

Infected females and males were released on a six-week old buffalo calf as part of a collaborative experiment with the sections of Animal Virology and Serology. Virus was detectable in the calf serum drawn three days and six days after releasing the ticks. The calf remained healthy but serum drawn on the 30th day after releasing the ticks neutralized 3.6 logs of KFD virus. During these 30 days, the rectal temperature of the calf varied from 99°F to 100.6°F. Serum samples from the calf drawn before releasing the ticks and seven days, 15 days, 20 days, 25 days, 30 days and 61 days after releasing the ticks, were tested by the HI test. Preserum and the seventh day serum were negative for KFD antibodies. Fifteenth and 20th day sera had HI antibody titres of 1:20 to KFD only. However, the titre had dropped to 1:10 by the 25th day and remained at this level up to the 61st day. It may be of interest to note that the serum drawn 30 days after releasing the ticks neutralized 3.6 logs of KFD virus while its HI antibody titre was only 1:10.

Eggs and larvae from infected female ticks were tested for the presence of virus by inoculation of the suspensions into mice, but so far there is no evidence for transovarial transmission of the virus.

A chick bitten by a nymph which had acquired the infection as a larva from an infected chick, was used as the donor for infecting a fresh batch of H. spinigera larvae by feeding. Blood drawn from the chick just before the larvae were released and titrated in adult mice after six days storage at -50°C had a titre of 2.6. Suspension of a fed larva made as soon as it had fed and dropped off from the chick and titrated after 124 days storage at -50°C had a titre $\geq$4.0. Suspensions of fed larvae in the moulting phase made ten days after the feed and titrated after 115 days storage at -50°C had a titre of 6.3. A total of 102 nymphs (68 of them dead) were tested in 12 pools by inoculation of the suspensions into adult mice, 20 to 56 days after the infecting feed. Only four pools representing 45 nymphs (27 of them dead) were positive, suggesting that the infection rate was probably low. The nymphs were released on 21, one-seven-day old chicks 24 to 60 days after the infecting feed and 13 to 46 days after emergence. Evidence of transmission was obtained in only four of the chicks.

4. ADMINISTRATIVE

4-1 Staff

4-1-1 Professional personnel

Charles R. Anderson, Staff Member, The Rockefeller Foundation.
July 15 to December 31.

P.N. Bhatt, Assistant Research Officer (ICMR) appointed Research Officer November 7.
Throughout the year.

M.S. Gokhale, Assistant Research Officer (RF)
January 1 to May 22.

T.B. Gokhale, Assistant Research Officer (ICMR)
Throughout the year.

K.G. Kulkarni, Assistant Research Officer (ICMR)
January 1 to May 22.

H.J. Mansharamani, Assistant Research Officer (ICMR)
Throughout the year.

M.N. Natu, Assistant Research Officer (ICMR)
Throughout the year.

K.M. Pavri, Assistant Research Officer (ICMR)
June 2 to December 31.

F.M. Rodrigues, Assistant Research Officer (ICMR)
Throughout the year.

K.V. Shah, Assistant Research Officer (ICMR) ~~appointed Research Officer~~ (ICMR) November 7.
November 7, to December 31.
(Was on special leave abroad upto November 6.)

Harold Trapido, Staff Member, The Rockefeller Foundation.
Throughout the year.

M.G.R. Varma, Research Officer (ICMR)
Throughout the year.

H.E. Webb, Temporary Staff Member, The Rockefeller Foundation.
January 4 to December 31.

Telford H. Work, Staff Member, The Rockefeller Foundation.
January 1 to August 9.

4-1-2 Sub-professional personnel

C.N. Dandawate, Research Assistant (ICMR).
Throughout the year.
(On deputation abroad from January 1 to April 20 for special training in vaccine production).

P.K. Rajagopalan, Research Assistant, (ICMR)
January 1 to May 24.
(On special leave abroad for training from May 25 to December 31.

N. Rishikesh, Research Assistant (RF)
January 1 to May 22.

Urmila Rau, Research Assistant (RF)
Throughout the year.

4-1-3 Roster of staff as of 31st December, 1958.

	Paid by	
	Indian Council of Medical Research	Rockefeller Foundation
RF staff members	-	3
Research Officers	3	-
Assistant Research Officers	5	-
Research Assistants	2	1
Senior Laboratory Assistants	4	5
Field Supervisor	1	-
Laboratory Assistants	6	-
Media Makers	9	4
Insect Collectors	2	12
Laboratory Attendants	8	2
Animal Attendants	13	6
Carpentry	2	1
Work-shop	1	1
Store	2	2
Secretary	-	1
Assistant Secretary	-	1
Book-keeper	-	1
Accounts Clerk	-	1
Stenographer	1	-
Steno-typists	1	1
Senior Clerk	1	-
Clerks	2	-
Laboratory Recorder	1	-
Assistant Laboratory Recorder	-	1
Librarian	-	1
Peons	2	-
Peon-cum-Van Driver	-	1
Van Drivers	3	3
Sweepers	7	4
Porter	1	-
Mali	1	-
Watchman	3	-
TOTAL	81	[illegible]

4-2 Budget

The VRC is jointly maintained by the Indian Council of Medical Research and the Rockefeller Foundation. Budget contributions are as follows, the ICMR contribution being shown for the budget in the fiscal year beginning 1 April 1958 and ending 31 March 1959, while the RF contribution is for the calendar year 1958:

(1) Budget allotment of the Indian Council of Medical Research for the VRC (Fiscal year 1 April 1958 to 31 March 1959)

I.	Pay	Rs. 1,63,280
II.	Dearness pay	39,045
III.	Dearness allowance	46,365
IV.	House rent allowance	19,630
V.	Compensatory allowance	7,265
VI.	Travelling allowance	18,000
VII.	Animals, their upkeep, automobile transport and stores and supplies	2,32,700
VIII.	Contingencies	30,000
IX.	Construction of building	1,07,735
	Total	Rs. 6,64,020

(2) Budget allotment of the Rockefeller Foundation to the VRC* (Calendar year 1 January to 31 December 1958).

I.	Salaries	Rs. 90,000
II.	Supplies and Equipment	2,61,489
III.	Operations	50,000
IV.	Contingencies	2,000
V.	Equipping new building	2,36,250
	Total	Rs. 6,39,739

* Excluding RF staff members.

4-3 Visitors during 1958

Dr. R.K. Anderson ..	The Rockefeller Foundation, New York.
Dr. Sef-El-Din El Arnaouti ..	Deputy Director Epidemic Control Section, M.P.H. Cairo, Egypt.
Dr. S. Creamer ..	Merck Sharp & Dohme, Philadelphia, Pa., U.S.A.
Prof. J.D.H. de Sa & 31 students..	Microbiology Department, Bhavan's College, Andheri, Bombay-41.
Dr. E. Eylan ..	Felix Public Health Laboratory, Tel Aviv, Israel.
Dr. D.C. Gajdusek ..	National Institutes of Health, Bethesda, Maryland, U.S.A.
Dr. M. Imanoedin ..	Health Services, East Java-Indonesia.
Prof. Otto Jirome ..	Prague, Czechoslovakia.
Dr. Y. Kameswara Rao & 28 students ..	M.P. College of Veterinary Science & Animal Husbandry, Mhow.
Dr. Masatsugu Kanamitsu ..	Sapparo Medical College, Japan.
Mr. M. S. Kannamwar ..	Minister of Public Health, Bombay.
Dr. A.K. Kapur ..	Zoological Survey of India, Calcutta.
Mr. D.P. Kapur ..	B.P. Division, Badshah Bag, Lucknow.
Dr. B.S. Keshavamurthy ..	Mysore Serum Institute, Bangalore.
Mr. Samuel McClenahan ..	Merck Sharp & Dohme, Philadelphia, Pa., U.S.A.
Dr. Jivraj N. Mehta ..	Minister of Finance, Bombay State.
Mr. Chamseddine M.H. Mofidi ..	Director, Institute of Malariology, Teheran University School of Medicine, Teheran, Iran.
Mr. Sheikh Ghulam Mohinuddin ..	Veterinary Laboratory, Srinagar.
Dr. R.S. Morison ..	The Rockefeller Foundation, New York
Prof. Dr. Stefan Nicolau ..	Rumanian Academy of Sciences, Bucharest, Rumania.
Dr. Vimol Notananda ..	Malaria Control Office, Chiengmai, Thailand.

Dr. H.C. Pant	..	Insect Physiologist, Indian Agricultural Research Institute, New Delhi.
Captain G.S. Perhar	..	Officer Commanding, Military Veterinary Hospital, National Defence Academy, Khadakvasla, Poona.
Dr. A.F. Pukhner	..	Institute of Poliomyelitis, Academy of Sciences, Moscow, U.S.S.R.
Dr. B.A. Rao	..	Malaria Institute of India, Delhi.
Dr. Morris Schaeffer	..	U.S.P.H.S. Communicable Disease Center. Virus Laboratory, Montgomery, Alabama, U.S.A.
Dr. Edwin W. Schultz	..	Stanford, California, U.S.A.
Dr. Sunthorn Srihongse	..	Department of Health, Bangkok, Thailand.
Dr. I. Tayaja	..	Department of Virology & Rickettsiology, National Institute of Health, Kamioseki, Shinagawa-Ku, Tokyo.
Major Tej Singh	..	Deputy Assistant Director of Remount Veterinary and Farms, Headquarters Bombay Area, Colaba, Bombay-5.
Dr. B.M. Thakral.	..	Research Officer, Indian Veterinary Research Institute, Izatnagar.
Dr. A. Tohibfadeh	..	Director General of the Ministry of Health, Teheran, Iran.
Mr. M. Usman	..	P.A. to Collector, Poona.
Dr. Term Vajrasthira	..	Malaria Control Office, Payao, Thailand.
Mr. N.N.S Verma	..	Assistant Disease Investigation Officer, North Bihar Range, Muzafarpur, Bihar.
Dr. J.W. Wright	..	World Health Organization, Geneva, Switzerland.

4-4 Training.

Facilities for training have been limited due to the physical lack of space in the present laboratory building but during the year several trainees were accommodated as follows:

Dr. S.N. Chatterjee, June 10 to December 9.	..	Calcutta School of Tropical Medicine (ICMR/RF Fellow)
Dr. Ambhan Dasaneyavaja, January 1 to March 31.	..	School of Medicine, Chulalongkorn Hospital, Bangkok, Thailand. (RF Fellow)
Dr. M.S. Jayadevaiah, May 15 to November 27	..	Mysore Department of Public Health.
Mr. A.P. Patil May 31 to November 27	..	Mysore Department of Public Health.
Dr. Dragoljub Sovrilich, October 7 to November 17.	..	Yugoslavia (Sponsored by the Ministry of Scientific Research and Cultural Affairs reciprocal research scheme).
Dr. S. Upadhyaya, May 15 to November 27.	..	Mysore Department of Public Health.

4-5 *Progress of new building*

During the year work continued on the construction of a new laboratory wing which will more than double the present working space which has for some time been woefully inadequate to the needs of the research and training program of the VRC. It is anticipated that the new wing will be ready for occupancy in May or June of 1959.

The new structure of three floors will house the virology, serology, Tissue Culture and Administrative Sections and also provide additional room for the mouse colony and infected experimental animals. There will also be space for the library, conference room and Zoological Sections. The bulk of the mouse colony and the Entomology Section will continue to occupy the present building. The removal of other sections to the new building will, however, release space for the installation in the Entomology Section of temperature, humidity and light controlled rooms where colonies of arthropods can be maintained for use in laboratory transmission work and studies on bionomics.

The new building is being constructed by the Indian Council of Medical Research with the Rockefeller Foundation providing funds for the central air conditioning plant and the scientific equipment.

4-4 Acknowledgements to cooperating agencies and collaborators.

Much that has been accomplished during the year has been made possible through the cooperation afforded the VRC by other agencies and individuals. It is with pleasure that the staff of the VRC acknowledges this help which it is hoped has aided in providing new knowledge of value to all.

While we list below those who have in various ways contributed to our efforts during the year, we wish to make first mention of the villagers of the KFD epidemic area who have with admirable patience served as valued informants, answered questions almost without end and donated the essential blood specimens which have made it possible to continue the development of the understanding of the natural history of the viral agent which has appeared in their midst.

INDIA

Mr. Humayun Abdulali	..	Bombay Natural History Society, Bombay - 6.
Dr. Riaz Ahmed	..	Medical Officer of Health, Mysore Department of Public Health, Sorab, Mysore State.
Mr. Salim Ali	..	Bombay Natural History Society, Bombay - 6.
American Consulate General	..	Bombay 26.
Dr. S.P. Aniker	..	KFD Special Officer, Mysore Department of Public Health, Sagar, Mysore State.
Dr. J.N. Berry	..	Professor of Medicine, Medical College, Nagpur.
Dr. P.M. Bhandarkar	..	Dean, Nagpur Medical College & Hospital, Nagpur.
Dr. J.K. Bhatnagar	..	Medical Officer of Health In-charge WHO Plague Project, Dehra Dun.
Dr. B.S. Bhatt	..	Special Malaria Officer, Government of Bombay, Rajkot, Saurashtra.
Dr. Mahendra J. Bhatt	..	Deputy Director of Public Health, Bombay, Rajkot, Saurashtra.
Dr. P.D. Bhave	..	Deputy Director of Public Health Services, Nagpur.
Mr. S.G. Bhogle	..	Wild Life Preservation Officer, Government of Bombay, Poona - 1.

B.J. Medical College and Sassoon Hospitals, Poona - 1.

Mr. Naikwadi	..	Superintendent, Manjri Agricultural School and Farm, Manjri, Poona.
Major-General Sarup Narain	..	Commandant, Armed Forces Medical College, Poona 1.
Dr. (Miss) Sharda Paul	..	Department of Virology and Pathology, King George Medical College, Lucknow.
Dr. T.B. Patel	..	Director of Public Health, Government of Bombay, Connaught House, Poona 1.
Captain G.S. Perhar	..	O.C. Veterinary Hospital, National Defence Academy, Khadakvasla.
Dr. G.S. Puri	..	Regional Botanist, Botanical Survey of India, Poona 1.
Dr. C.M. Rangan	..	Head, Department of Pathology, M.G. Memorial Medical College, Indore, M.P.
Dr. S.H. Rahaman	..	Medical Officer of Health (KFD), Mysore Department of Public Health, Sagar, Mysore State.
Dr. (Mrs.) Kamal J. Ranadive	..	Indian Cancer Research Centre, Bombay - 12.
Dr. D.L.N. Murty Rao	..	Deputy Superintendent, Mental Hospital, All India Institute of Mental Health, Bangalore 2.
Mr. P.V.R. Rao, ICS	..	Chief Secretary, Government of Mysore, Bangalore.
Dr. R. Lakshmana Rao	..	Assistant Surgeon, Sagar Combined Hospital, Mysore Department of Medical Services, Sagar, Mysore State.
Dr. T.R. Rao	..	Assistant Director of Public Health (Malaria), Bombay State, Poona 1.
Dr. R. Seshagiri Rao	..	Director of Public Health, Government of Mysore, Bangalore.
Dr. M. Shama Rao	..	District Health Officer, Mysore Department of Public Health, Shimoga.
Dr. V.N. Rao	..	Assistant Director of Public Health (Epidemiology), Bombay State, Poona 1.

Dr. Raymond C. Gochenour .. Biological Products Research Laboratory, Division of Immunology, Walter Reed Army Institute of Research, Washington, D.C., U.S.A.

Dr. H. Hoogstraal .. NAMRU-3, Cairo, Egypt.

Dr. Makram Kaiser .. NAMRU-3, Cairo, Egypt.

Dr. E.F. Knipling .. U.S. Department of Agriculture, Entomology Research Division, Beltsville, Maryland, U.S.A.

Mr. G.M. Kohls .. Rocky Mountain Laboratory, Hamilton, Montana, U.S.A.

Professor E.N. Levkovich .. Ivanovsky Virology Institute, Moscow, U.S.S.R.

Professor P.A. Petrischeva .. Gamalaya Institute of Epidemiology and Microbiology, Moscow, U.S.S.R.

Professor A.A. Smorodintsev .. Institute of Experimental Medicine. Leningrad, U.S.S.R.

Dr. Alan Stone .. U.S. National Museum, Washington, D.C., U.S.A.

Dr. Arne Svedmyr .. Central Bacteriological Laboratory of Stockholm City, Stockholm, Sweden.

Dr. T. Vaithianathan .. Honorary Secretary, Medical Research Group, Colombo 3, Ceylon.

Dr. Bernard Weitz .. Lister Institute of Preventive Medicine, Elstree, England.

5. *PUBLICATIONS DURING THE YEAR*

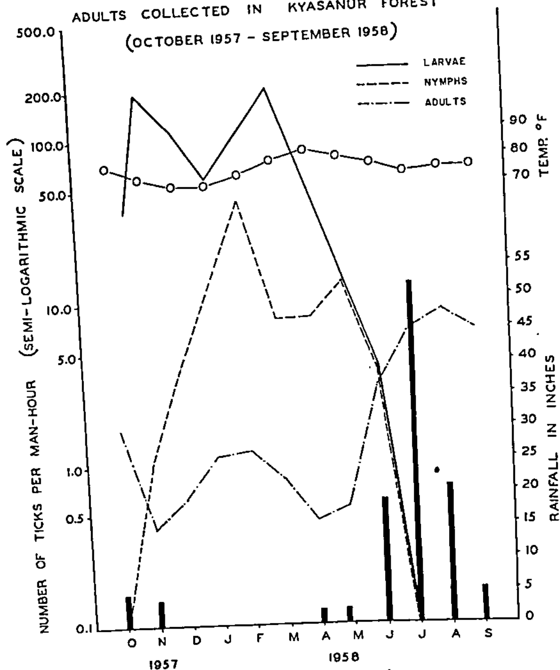
1. Work, T.H. 1958. Virological aspects of Kyasanur Forest Disease. J. Ind. Med. Assoc., 31: 111-113.

2. Work, T.H. 1958. Russian spring-summer virus in India. Kyasanur Forest Disease. Prog. Med. Virology, 1: 248-277.

6. ADDENDUM

Inadvertently omitted from the list of the professional staff (Section 4-1-1) is the name of Dr. (Miss) Sheila Pereira, Pediatrics Registrar, Christian Medical College Hospital, Vellore, January 1 - June 30. (RF). In this section also there should be added to the notation accompanying the name "Harold Trapido", "abroad from June 16 to October 14".

FIGURE 1 (See 2-1-6-1)



M1406
6511

FIGURE 2 (Sec 3-1-3)

VIRUS TITRES IN BLOOD OF 4 CONTROL MONKEYS INOCULATED WITH KFD VIRUS

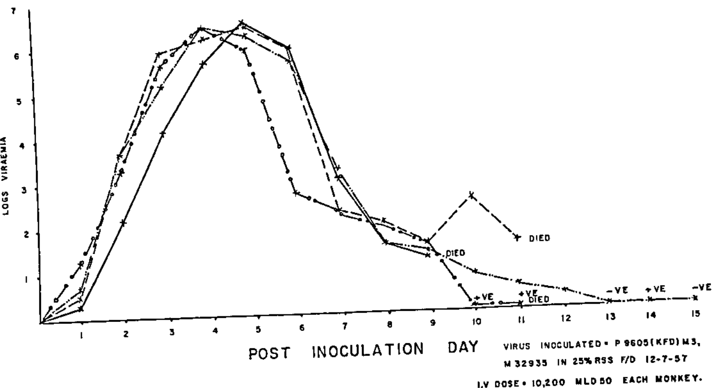


FIGURE 4 (See 3-1-4)

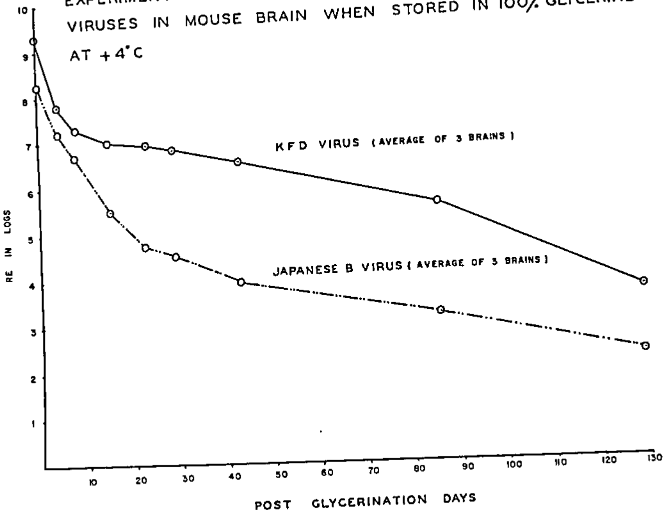


TABLE 1 (See 2-1-1)

Diagnosis of Illnesses Investigated in 1958 by Month of Onset (KFD)

Category	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
KFD	1	8 (5)	13 (8)	15 (9)	8 (7)	1 (1)		-				1 (1)	47 (31)
Not KFD	2	20	16	27	25	16	10	13	22	13	12	2	178
Undetermined	0	4	4	8	6	3	1	1	1	1	6	7	42
Total	3	32	33	50	39	20	11	14	23	14	18	10	267

Figures within brackets represent cases from whom virus was isolated.

TABLE 2 (See 2-1-2-1)

HI Antibody Response to the Vaccine

Category	Number	No Rise in HI Antibodies	Rise in HI Antibodies			
			Tube 1	Tube 2	Tube 3	Tube 4
With previous Group B HI Antibodies *	179	85	53	25	12	2
Percent	100	47.5	29.5	14.0	6.7	1.1
Without previous Group B HI Antibodies	217	193	13	4	3	4
Percent	100	88.9	6.0	1.8	1.4	1.9

* In two instances (1.1%) the post serum showed one tube less than pre serum.

* * * * * *

TABLE 3 (See 2-1-2-1)

CF Antibody Response to the Vaccine

Category	Number	No Rise in CF Antibodies	Rise in CF Antibodies			
			Tube 1	Tube 2	Tube 3	Tube 4
With previous Group B CF Antibodies	66	66	0	0	0	0
Percent	100	100	0	0	0	0
Without previous Group B CF Antibodies	331	331	0	0	0	0
Percent	100	100	0	0		

TABLE 4 (See 2-1-2-1)

Neutralising Antibody Response to the Vaccine *

	Run No.	Dose Log LD_{50}	Number of pairs	Protection	Partial Protection	No Protection
I	590	3.2	27	0	6	21
		Percent	100	0	22	78
II	592	0.8	13	5	5	3
	593	0.7	21	7	8	6
	595	1.0	16	9	3	4
		Total	50	21	16	13
		Percent	100	42	32	26

* The neutralisation tests were done intraperitoneally in 2-day old mice.

* * * * * *

TABLE 5 (See 2-1-2-1)

Comparison of Neutralising Antibody Response Between Vaccinees With and Without Group B HI Antibodies in the Pre Vaccination Serum

Category	Total	Protection	Partial Protection	No Protection
With previous Group B HI Antibodies	28	10	11	7
Percent	100	36	39	25
Without previous Group B HI Antibodies	22	11	5	6
Percent	100	50	23	27
Total	50	21	16	13

TABLE 6 (See 2-1-3)

Monthly Distribution of Reported Monkey Deaths During 1958
According to Estimated Date of Death.

1958	*Presbytis entellus*	*Macaca radiata*	Species not Determined	Total
January	6	1		7
February	26	1		27
March	16		1	17
April	6			6
May	10		2	12
June	9		8	17
July	6	1		7
August	1			1
September	2	1		3
October	6		4	10
November	3			3
December	7			7
Total	98	4	15	117

TABLE 8 (See 2-1-4) Contd.

Localities	RRW	RRR	RB	SM	FT	MB	Misc	Total
	P/PP/Tot	P/PP/Tot	P/PP/Tot	P/PP/Tot	P/PP/Tot	P/PP/Tot	P/PP/Tot	P/PP/Tot
Kuppe*	0/ 0/ 10	0/ 0/ 7	0/ 0/ 2	2/ 0/ 9	0/ 0/ 0	0/ 0/ 0	0/ 0/ 0	2/ 0/ 28
Maduba Siddapur	0/ 3/ 17	0/ 0/ 0	0/ 0/ 0	0/ 0/ 2	5/ 0/ 10	0/ 2/ 7	0/ 0/ 6	5/ 5/ 42
Ulavi-Kaisodi	0/ 1/ 29	0/ 0/ 0	0/ 0/ 6	0/ 0/ 1	0/ 0/ 0	0/ 0/ 1	0/ 0/ 0	0/ 1/ 37
TOTAL	0/ 6/124	0/ 0/ 9	1/ 1/ 23	4/ 1/ 31	5/ 2/ 28	1/ 2/ 17	0/ 0/ 11	11/12/243

RRW = Rattus rattus wroughtoni

RRR = Rattus rattus rattus

RB = Rattus blanfordi

P = Positive

SM = Suncus murinus

FT = Funambulus tristriatus

MB = Mus booduga

PP = Partial protective (see explanation in Section 2-1-4)

* Localities at which human cases of KFD have not been noted.

Numbers of *Haemaphysalis* and *Ixodes* Larvae, Nymphs and Adults Collected per man-hour from Drags in Kyasanur Forest. Oct. 57 - Sept. 58.

Month	Rainfall (inches)	Mean-temp (° F)	*Haemaphysalis*			*Ixodes*		
			Larvae	Nymphs	Adults	Larvae	Nymphs	Adults
October 1957	4.92	78.6	26.61	0.1	1.67	0.53	<0.1	0
November 1957	3.89	73.8	186.1	1.06	0.41	0.59	0	0
December 1957	Nil	71.2	117.1	4.07	0.5	2.36	<0.1	0
January 1958	Nil	71.2	55.85	11.44	1.12	0	0	0
February 1958	Nil	74.8	100.45	41.1	1.2	0.1	0.25	0
March 1958	Nil	79.6	202.5	7.71	0.8	<0.1	0	0
April 1958	1.97	82.6	52.28	8.08	0.2	10.3	0	0
May 1958	2.09	81.0	15.3	12.92	0.54	51.07	0	0
June 1958	19.13	77.6	3.96	3.68	2.97	56.31	0	0
July 1958	51.65	74.0	0	0	6.5	0	0	0
August 1958	21.23	74.9	0	0	8.2	0	0	0
September 1958	5.17	75.3	0	0	6.5	0	0	0

TABLE 10 (See 2-1-6-1)

Numbers of Haemaphysalis spinigera taken in ground collections in Kyasanur Forest, Kannur and Hosur for the period June-September 1958

	Total number of adults of all species	*H. spinigera*		
		Number	%	per mh
Kyasanur Forest	966	378	39.1	2.5
Kannur	695	632	90.9	4.5
Hosur	1253	1151	91.9	8.6

TABLE 11 (See 2-1-6-1)

Comparison of the adult tick fauna in Kyasanur Forest, Kannur and Hosur, based on ground collections during the period June-September 1958.

Species	Per cent collection		
	Kyasanur Forest (Total coll: 966 adults)	Kannur Forest (Total coll: 695 adults)	Hosur Forest (Total coll: 1253 adults)
Haem. formosensis	1.7	0	0
Haem. papuana	38.6	1.9	2.6
Haem. turturis	14.6	4.6	4.7
Haem. cuspidata	0.4	0.1	0.5
Haem. aculeata	0.6	0	<0.1
Haem. spinigera	39.1	90.9	91.9
Haem. minuta	0.4	0	0
Haem. cornigera	3.2	1.9	0.2
Rhip. haemaphysaloides	0	0.1	<0.1
Dermacentor auratus	0.9	0.1	0
Amblyomma integrum	0.4	0	0

TABLE 12 (See 2-1-6-1)

Number of different species of Haemaphysalis adults taken per man-hour in ground collections in Kyasanur Forest during the period October 57 - September 1958.

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
H. formosensis	<0.1	0.1	0	0.5	0.2	0.2	0	0	0	<0.1	<0.1	0.3
H. papuana	0.5	0	0	0	0.2	0	0.1	0.2	1.8	2.7	3.4	1.8
H. turturis	0.6	0.2	0.5	0.4	0.6	0.6	<0.1	0	0.3	0.9	1.3	1.1
H. cuspidata	0	<0.1	0	0	0	0	<0.1	0	0	<0.1	<0.1	0
H. aculeata	<0.1	0	0	0	<0.1	0	0	0	0	<0.1	0	0.1
H. spinigera	0.5	<0.1	0	0.2	<0.1	0	<0.1	0.4	0.8	2.8	3.2	2.9
H. wellingtoni	0	0	0	0	0.1	0	0	0	0	0	0	0
H. minuta	0	0	0	0	0	0	0	0	<0.1	<0.1	0	<0.1
H. cornigera	<0.1	0	0	0	<0.1	0	0	0	<0.1	0.1	0.3	0.3
Total	1.7	0.4	0.5	1.1	1.2	0.8	0.2	0.6	2.9	6.5	8.2	6.5

TABLE 13 (See 2-1-6-1)

Species of adult Haemaphysalis taken in ground collections in Kyasanur Forest during October 1957 to September 1958, in order of abundance.

Species	Total number collected	Number of months in the 12-month period in which collected.
H. spinigera	438	10
H. papuana	435	8
H. turturis	227	11
H. formosensis	27	10
H. cornigera	27	6
H. aculeata	7	4
H. cuspidata	5	4
H. minuta	4	3
H. wellingtoni	2	1

TABLE 14 (See 2-1-6-2)

Infestation rate of small mammals with ticks in Kyasanur, Kannur and Hosur forests from Oct 57-Sept 58.

Month & Year	Total examined	Number positive	Infestation rate %
October 1957	76	27	35.5
November 1957	109	34	31.2
December 1957	No trapping done		
January 1958	35	21	60.0
February 1958	68	38	55.9
March 1958	8	5	62.5
April 1958	41	16	39.0
May 1958	81	65	80.2
June 1958	55	30	54.5
July 1958	75	29	38.7
August 1958	76	28	36.8
September 1958	49	37	75.5
	673	330	49.0

TABLE 15 (See 2-1-6-2)

Infestation Rate of Different Species of Small Mammals with Ticks in Kyasanur, Kannur and Hosur Forests from October 1957 to September 1958.

Species	Number Examined	Number with Ticks	Infestation rate %
Rattus rattus wroughtoni	407	209	51.4
Rattus rattus rufescens	15	6	40.0
Rattus blanfordi	126	64	50.8
Mus booduga	28	3	10.7
Tatera indica hardwickei	5	2	40.0
Funambulus tristriatus tristriatus	32	23	71.9
Suncus murinus	59	22	37.3

TABLE 16 (See 2-1-6-2)

Numbers of Haemaphysalis and Ixodes Larvae, Nymphs and Adults Collected per infested small mammal in Kyasanur, Kannur and Hosur Forests from October 1957 - September 1958.

Month & Year	Total number positive for ticks	Ixodes			Haemaphysalis		
		Larvae	Nymphs	Adults	Larvae	Nymphs	Adults
October 1957	27	2.3	2.7	0	0.6	0.2	0
November 1957	34	1.5	1.6	0	0.4	0.3	0
December 1957	No trapping done						
January 1958	21	<0.1	0.7	0	1.5	3.1	0
February 1958	38	0.4	1.0	0	0.1	2.2	0
March 1958	5	0.2	0.2	0	0	2.4	0
April 1958	16	6.0	0.1	0	0	0.8	0
May 1958	65	19.0	<0.1	<0.1	<0.1	0.4	0
June 1958	30	7.3	0.6	0	<0.1	<0.1	0
July 1958	29	2.3	2.0	0.1	0	0	0
August 1958	28	5.3	4.9	<0.1	0	0	0
September 1958	14	4.4	0.9	0	0	0	0
Total number		1955	413	8	71	213	0

TABLE 17 (See 2-1-2-2)

Sex Distribution of 1126 Vaccinees of 14 Villages

Sl. No.	Village	Male	Female	Total
1	Horabailu	56	38	94
2	N.H. Hosur	45	37	82
3	Kanahalli & Koppalagadde	33	21	54
4	Maisavo	21	8	29
5	Dugur & Bhadrapur	52	40	92
6	Bhadrapura	19	13	32
7	Padavagodu	71	55	126
8	Harur	50	42	92
9	Hessare	68	40	108
10	Barur	32	36	68
11	Kumbatti	41	24	65
12	Maduba Siddapur	60	41	101
13	Kaisodi	87	52	139
14	Hunavalli	26	18	44
	Total	661	465	1126
	Percent	59	41	100

TABLE 18 (See 2-1-2-2)

Age and Sex Distribution of Laboratory Proved Cases in 1957 and 1958.

	Age Group								Total
	0-4	5-9	10-14	15-19	20-29	30-39	40-49	Over 50	
1957									
Male	0	0	4	7	12	14	3	2	42
Female	0	0	1	0	3	1	1	0	6
Total	0	0	5	7	15	15	4	2	48
1958									
Male	0	0	6	6	10	15	2	2	41
Female	0	0	1	0	3	1	1	0	6
Total	0	0	7	6	13	16	3	2	47
1957-1958									
Male	0	0	10	13	22	29	5	4	83
Female	0	0	2	0	6	2	2	0	12
Total	0	0	12	13	28	31	7	4	95

* * * * * * *

TABLE 19 (See 2-1-2-2)

Age and Sex Distribution of 1126 Vaccinees of 14 Villages

	Age Group									Total
	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-59	Over 60	
Male	4	102	84	57	141	136	79	40	18	661
Female	2	93	72	43	101	73	45	26	10	465
Total	6	195	156	100	242	209	124	66	28	1126

[illegible]

Incidence of KFD in 1957 & 1958

Year	Number of villages affected*	Population	Total cases reported	Total cases examined satisfactorily	Proved cases	Estimated number of cases in those reported
1957	20	3367	466		48	
1958	15	2605	281	225	47	± 60

* Those with at least one laboratory proved clinical case. Nine villages affected in both years, 11 in 1957 only and six in 1958 only.

* * * * * * *

TABLE 21 (See 2-1-2-2)

Speculation on Number of Cases in Males Over Ten who have received all Three Doses of Vaccine if (a) Attack Rate 5% for Males Over Ten and (b) Vaccine Totally Ineffective.

	Number of villages	Population	Estimated vaccinees 55% of popu.	Estimated having 3 doses 26% of popu.	Males over 10 with 3 doses 50% of column 4	Expected cases in males over 10
(a) If cases in each village infected in 56 and/or 57 and/or 58	27	4191	2310	1092	546	27
(b) If cases in each village vaccinated	89	15747	8635	4082	2041	102
(c) If disease as in 1958	15	2605	1430	676	338	17

TABLE 22 (See 2-5-1)

Incidence of CF and HI antibodies to Group B viruses in six localities of Saurashtra

Locality	Sex	HI 0-14	HI 15 +	HI Total	HI % Positive	CF 0-14	CF 15 +	CF Total	CF % Positive
..ad	Male	4/12	27/27	31/39	79.5	0/12	11/27	11/39	28.2
	Female	4/ 6	7/ 7	11/13	84.6	2/ 6	2/ 7	4/13	30.7
	Total	8/18	34/34	42/52	80.7	2/18	13/34	15/52	28.8
	% Positive	44.4	100	80.7		11.1	38.2	28.8	
..eshwar	Male	0/ 4	4/12	4/16	25.0	0/ 4	1/11	1/15	6.7
	Female	0/ 0	0/ 1	0/ 1	0	0/ 0	0/ 1	0/ 1	0
	Total	0/ 4	4/13	4/17	23.5	0/ 4	1/12	1/16	6.2
	% Positive	0	30.7	23.5		0	8.3	6.2	
..bala	Male	2/ 7	11/13	13/20	65.0	0/ 7	5/13	5/20	25.0
	Female	0/ 3	3/ 9	3/12	25.0	0/ 3	1/ 9	1/12	8.3
	Total	2/10	14/22	16/32	50.0	0/10	6/22	6/32	18.7
	% Positive	20	63.6	50.0		0	27.3	18.7	
..a	Male	4/ 6	19/21	23/27	85.2	2/ 6	11/21	13/27	48.1
	Female	6/11	13/13	19/24	79.2	3/11	5/13	8/24	33.3
	Total	10/17	32/34	42/51	82.3	5/17	16/34	21/51	41.2
	% Positive	58.8	94.1	82.3		29.4	47.0	41.2	

TABLE 22 (contd.) (See 2-5-1)

ity	Sex	HI 0-14	HI 15 +	HI Total	HI % Positive	CF 0-14	CF 15 +	CF Total	CF % Positive
tpura	Male	2/ 7	22/25	24/32	75.0	0/ 6	7/22	7/28	25.0
	Female	5/ 7	8/11	13/18	72.2	0/ 4	1/ 8	1/12	8.3
	Total	7/14	30/36	37/50	74.0	0/10	8/30	8/40	20.0
	% Positive	50.0	83.3	74.0		0	26.6	20.0	
Forest	Male	3/ 3	15/25	18/28	64.3				
	Female	0/ 1	5/ 9	5/10	50.0				
	Total	3/ 4	20/34	23/38	60.5				
	% Positive	75.0	58.8	60.5					
TOTAL	Male	15/39	98/123	113/162	63.5	2/35	35/94	37/129	28.6
	Female	15/28	36/50	51/ 78	65.3	5/24	9/38	14/ 62	22.6
	Total	30/67	134/173	164/240	68.3	7/59	44/132	51/191	26.7
	% Positive	44.8	77.4	68.3		11.9	33.3	26.7	

TABLE 23 (See 2-5-1)

Incidence of neutralising antibodies to KFD in six localities of Saurashtra

Locality	Total No.	Sex	Age 0-14	Age 15 +	Total
Bhanvad	52	Male	0/12	0/27	0/39
		Female	0/ 6	1/ 7	1/13
			0/18	1/34	1/52
illeshwar	17	Male	0/ 4	1/12	1/16
		Female	0/ 0	0/ 1	0/ 1
			0/ 4	1/13	1/17
mbala	32	Male	0/ 7	3/13	3/20
		Female	0/ 3	0/ 9	0/12
			0/10	3/22	3/32
iyana	51	Male	0/ 6	9/21	9/27
		Female	0/11	5/13	5/24
			0/17	14/34	14/51
atpura	50	Male	0/ 7	1/25	1/32
		Female	0/ 7	2/11	2/18
			0/14	3/36	3/50
Gir Forest	38	Male	0/ 3	1/25	1/28
		Female	0/ 1	1/ 9	1/10
			0/ 4	2/34	2/38
Total	240	Male	0/39	15/123	15/162
		Female	0/28	9/ 50	9/ 78
			0/67	24/173	24/240
		% Positive	0	13.9	10.0

TABLE 24 (See 2-5-1)

CF and HI results on Sera Positive to KFD in Neutralisation Test.

S. No.	P. No.	Age/Sex	Locality	Occupation	HI				CF						Remarks
					JBE	E101	Tr1751	KFD	JBE	DN1	G2266	E 101	Tr1751	KFD	
1.	P 14078	35/F	Bhanvad	Chamar	3	4	4	4	<4		<4	<4	<4	<4	
2.	P 14135	35/M	Killeshwar	Cowherd	6	6	8	6	12	4	16	<4	32	4	
3.	P 14148	25/M	Khambala	Servant	2	3	3	3	<4		<4	<4	<4	<4	
4.	P 14151	50/M	Khambala	Farmer	0	0	0	1	<4		<4	<4	<4	<4	
5.	P 14159	45/M	Khambala	Service	4	4	5	5	<4		<4	<4	4	<4	
6.	P 14189	22/M	Kutiana	Labourer	3	3	6	3	<4		<4	<4	4	<4	
7.	P 14192	40/F	Kutiana	Sweeper	3	3	6	3	<4		<4	<4	<4	<4	
8.	P 14198	37/M	Kutiana	-	7	6	8	5	8	16	<4	<4	16	<4	
9.	P 14201	25/F	Kutiana	-	4	4	5	3	<4		<4	<4	4	<4	
10.	P 14203	50/F	Kutiana	Woodcutter	7	7	8	6	<4		<4	<4	12	<4	
11.	P 14204	80/F	Kutiana	Woodcutter	5	4	6	4	<4		<4	<4	4	<4	
12.	P 14218	16/M	Kutiana	Student	5	5	8	5	[illegible]	4	<4	<4	16	4	
13.	P 14222	15/M	Kutiana	Student	4	4	4	0	<4		<4	<4	<4	<4	
14.	P 14223	40/F	Kutiana	Sweeper	4	4	7	4	<4		<4	<4	<4	<4	
15.	P 14224	30/M	Kutiana	Sweeper	7	6	9	6	16		<4	<4	32	16	
16.	P 14225	30/M	Kutiana	Sweeper	4	4	6	4	<4		<4	<4	4	<4	
17.	P 14226	40/M	Kutiana	Sweeper	3	3	5	3	<4		<4	<4	<4		

TABLE 24 (contd.) (See 2-5-1)

S. No.	P. No.	Age/Sex	Locality	Occupatio	HI JBE	HI E101	HI Tr1751	HI KFD	CF JBE	CF DNI	CF G2266	CF E101	CF Tr1751	CF KFD	Remarks
18.	P 14227	22/M	Kutiana	Sweeper	0	1	3	0	<4		<4	<4	<4	<4	
19.	P 14228	17/M	Kutiana	Sweeper	5	5	7	5	4	16	4	<4	32	<4	
20.	P 14248	60/M	Daulatpura	Woodcutter	0	0	0	0			ac	ac	ac	ac	ac
21.	P 14250	60/F	Daulatpura	Woodcutter	5	5	8	5	<4		<4	<4	4	<4	
22.	P 14269	15/F	Daulatpura	Woodcutter	0	0	0	3	<4		<4	<4	<4	<4	
23.	P 14288	45/M	Gir Forest	Cowherd	0	0	0	1	-		-	-	-	-	ac Dudhala
24.	P 14310	30/F	Gir Forest	Farmer	3	3	5	4	<4		<4	<4	<4	<4	Sirvan

TABLE 25 (See 2-5-2)

Comparison of tick infestation of goats, cattle and buffaloes in Saurashtra with *Hyalomma* and *Haemaphysalis* ticks.

Animals	Total number of ticks collected	*Hyalomma*	*Haemaphysalis*	Others
Goats	128	24 %	75 %	1.0 %
Cattle	139	84 %	4 %	12 %
Buffaloes	76	89 %	9 %	1 %
Total	343	63 %	32 %	5 %

TABLE 26 (See 2-6)

HI and CF test results on sera from children of Ramtek, Bombay State

Age	Number positive/Number examined	
	HI	CF
6	4/4	3/4
7	5/5	3/5
8	8/8	5/8
9	9/9	8/8
10	5/5	4/4
Total	31/31	23/29
Percent	100	80

TABLE 27 (See 2-7)

CF and HI Results on Sera from Indore and Vicinity.

Locality	Total	Number positive by HI	Per cent positive by HI	Number positive by CF	Per cent positive by CF	Probable infection of CF positives			
						WN-JBE	DN II	KFD	Undetermined
Mandu	50	22	44 %	1	2 %	1	0	0	0
Bherdon Ghat	6	1	16 %	0	0	0	0	0	0
Bai	11	6	54 %	1	9 %	0	1	0	0
Choral	19	12	63 %	2	10 %	1	1	0	0
Hatod	40	16	40 %	6	15 %	0	5	0	1
Indore.	29	24	83 %	7	24 %	2	5	0	0
Total	155	82	53 %	17	11 %	4	12	0	1

TABLE 28 (See 3-1-1)

Viruses Isolated from Ticks Inoculated from Shimoga District from January to December 1958. (Unidentified, not KFD).

Sr. No.	VRC Number	Source	Locality	Mouse Pathogenicity Infant	Mouse Pathogenicity Adult	Re-isolation	Remarks
1	G 21850	Haemaphysalis sp. 6, 1 N, 26.2.58 Ectoparasitic on RRW (W 2262)	Jimanur	+	-	0	- Group B with quick antigen
2	G 23531	Haemaphysalis sp. 4, 1 N, 28.3.58 Ectoparasitic on RRW (W 2511)	Barage	+	-	-	
3	G 23582	Haemaphysalis type 4, 25 L,3.4.58 From ground drags.	Barage Station 2.	+	?	-	
4	G 23708	Haemaphysalis type 4, 50 L,3.4.58 From ground drags.	Barage Station 2.	+	-	-	- Group B with quick antigen
5	G 23764	Haemaphysalis sp., 1 L, 16.4.58 Ectoparasitic on Macaca radiata (W 2735)	Maduba Siddapur	+	-	-	
6	G 23797	Haem. wellingtoni, 2 N, 18.4.58 Ectoparasitic on Gallus sonnerati (F 4914)	Ulvi-Sorab Road 8/13	+	-	+	- Group B with quick antigen
7	G 24999	Haem. bispinosa, 1 F, 20.6.58 Ectoparasitic on cow calf.	Barage	+	-	0	Virus lost from glycerinated brain
8	G 26622	Haem. spinigera, 5 N, 4.8.58. From leaves.	Barage Station 2.	+	+	0	
9	G 26721	Ixodes sp., 1 L, 6.8.58. Ectoparasitic on RRW (W 4485)	Hosur	+	-	+	
10	G 26962	Ixodes sp. 1, 2 N, 9.9.58. From ladder drags.	Barage	+	-	0	- Group B with quick antigen
11	G 26971	Haem. papuana, 1 F, 9.9.58 From leaves.	Barage, Station 2.	+	-	-	

Sr. No.	VRC Number	Source	Locality	Mouse Pathogenicity Infant	Mouse Pathogenicity Adult	Re-isolation	Remarks
12	G 26981	Haem. spinigera, 3 M + F, 4.9.58. From leaves.	Kannur Station 3.	+	0	0	
13	G 27004	Haem. turturis, 4 M + F, 8.9.58. From leaves.	Hosur Station 2.	+	0	-	- Group B with quick antigen
14	G 27006	Ixodes sp., 1 L, 3.9.58. Ectoparasitic on RRW (W 4870)	Barage	+	-	-	
15	G 27053	Haem. spinigera, 25 M + F, 15.9.58. From leaves.	Barage Station 3	+	+	-	- Group B with quick antigen
16	G 27056	Haem. cornigera, 2 F, 15.9.58. From leaves.	Barage Station 3	+	-	-	
17	G 27062	Haem. spinigera, 1 F, 20.9.58. From leaves.	Kannur Station 1.	+	-	0	
18	G 27081	Ixodes sp. 1 L, 10.9.58. Ectoparasitic on RB (W 4960)	Kannur	+	0	-	- Group B with quick antigen
19	G 27119	Haemaphysalis sp. 2, 3 N, 13.10.58. From ground drags.	Barage Station 3.	+	-	-	
20	G 27153	Haem. cuspidata, 3 N, 29.9.58. From ground drags.	Barage Station 3.	+	-	-	- Group B with quick antigen
21	G 27158	Ixodes sp., 18 L, 29.9.58. From ground drags.	Barage Station 2.	+	-	-	- Group B with quick antigen
22	G 27163	Ixodes sp., 1 L, 6.10.58 From ground drags.	Barage Station 2	+	0	0	
	G 27166	Haem. turturis, 1 M, 6.10.58.	Barage Station 3	+	-	0	

TABLE 28 (contd) (See 3-1-1)

Sr. No.	VRC Number	Source	Locality	Mouse Pathogenicity		Re-isolation	Remarks
				Infant	Adult		
24	G 27264	Dermacentor sp. 2 N, 17.11.58. From ground drags.	Barage Station 2	+	-	-	
25	G 27333	Haem. spinigera, 56 N, 2.12.58 From ground drags.	Barage Stations 1 & 2	+	+	0	- Group B with quick antigen

+ Positive
- Negative
0 Not done

RRW Rattus rattus wroughtoni
RB Rattus blanfordi

* * * * *

TABLE 29 (See 3-1-1)

Viruses Isolated from Humans from Shimoga District from January to December 1958 (Not KFD).

Sr. No.	VRC Number	Source	Locality	Mouse Pathogenicity		Re-isolation	Remarks
				Infant	Adult		
1	P 15831	Human, M/30, 23.3.58. 2nd day of illness. Blood.	Dugur	+	-	-	
2	P 16385	Human, M/30, 15.4.58. 4th day of illness. Blood.	Maduba Siddapur	+	-	-	

+ Positive
- Negative

TABLE 30 (See 3-1-1)

Viruses Isolated from Mosquitoes Inoculated from North Arcot District from January to December 1958

Sr. No.	VRC Number	Source	Locality	Mouse Pathogenicity		Re-isol-ation	Remarks
				Infant	Adult		
1	G 22086	Culex vishnui, 150 F 19.6.58	Sathuperi, Outdoor resting	+	+	-	West Nile by CF block titration
2	G 25484	Culex fuscocephalus, 154 F 10.9.58	Kammavanpeth, Bullock biting	+	+	-	- Group B with quick antigen
3	G 25870	Culex whitmorei, 91 F 3.11.58	Devanampeth, Indoor resting	+	+	+	Group B by CF
4	G 25969	Culex vishnui, 34 F 20/21.11.58	Devanampeth, Bullock biting	+	+	-	- Group B with quick antigen
5	G 29842	Culex vishnui, 150 F 27.11.58.	Sathuperi, Outdoor resting	+	+	-	- Group B with quick antigen
6	G 29932	Culex vishnui, 150 F 9.12.58	Devanampeth, Outdoor resting	+	+	-	
7	G 29934	Culex whitmorei, 27 F 9.12.58.	Devanampeth, Outdoor resting	+	+	-	

+ Positive
- Negative

Viruses Isolated from Humans from Vellore Area from January to December 1958.

Sr. No.	VRC Number	Source	Locality	Mouse Pathogenicity		Re-isol-ation	Remarks
				Infant	Adult		
1	P 20173	Human, 10/M, 13.10.58. 5th day of illness. Brain.	Devanampeth	+	+	-	Japanese B by CF block titration
2	P 20458	Human, 4/M, 19.11.58. Brain.	?	+	+	0	+ Group B with quick antigen
3	P 20778	Human, 13/M, 12.12.58 3rd day of illness. Brain	Thenangoor (Wandiwash)	+	+	0	

* * * *

TABLE 32 (See 3-1-1)

Viruses Isolated from Mammals from Khadakvasla-Poona

Sr. No.	VRC Number	Source	Locality	Mouse Pathogenicity		Re-isol-ation	Remarks
				Infant	Adult		
1	W 2305	Horse serum, 7.3.58	Khadakvasla	+	+	-	- Group B with quick antigen
2	W 2330	Horse kidney, 8.3.58	Khadakvasla	+	+	+	- Group B with quick antigen
3	W 2415	Dog brain, 16.3.58	Khadakvasla	+	+	+	Negri body + rabies

\+ Positive

TABLE 33 (See 3.2.4)

Circulating virus in Rattus rattus wroughtoni and Rattus blanfordi inoculated intraperitoneally with the [illegible] strain of [illegible] virus.

No.	Sex	Post-inoculation day								
		1	2	3	4	5	6	7	8	9
T 1	M	NB	≤0.9	NB	NB	≤0.7	NB	NB	NB	-ve
T 2	F	NB	≤0.8	NB	NB	-ve	NB	NB	NB	-ve
T 3	F	NB	NB	-ve	NB	NB	?	NB	NB	-ve
T 4	F	NB	NB	≤1.0	NB	NB	2.4	NB	NB	-ve
T 5	M	NB	NB	1.5	NB	NB	-ve	NB	NB	-ve
T 6	M	NB	NB	NB	-ve	NB	-ve	NB	NB	-ve Died
T 7	[illegible]	NB	NB	NB	1.5	NB	≤0.8	NB	NB	-ve
TB1	F	NB	2.0	NB	4.8	NB	≤0.6	NB	NB	-ve
TB2	M	NB	NB	-ve	NB	-ve	NB	-ve	NB	-ve

T Rattus rattus wroughtoni

TB Rattus blanfordi

NB Not bled

TABLE 34 (See 3-4-4-3)

Persistence of KFD virus in *A. albopictus* following an infecting meal.

Virus strain	Donor animal	Circulating virus titer	Interval after feed upto which virus is detectable in mosquitoes
G 11338	Chick 1-2 days old	Detect. virus in 10^{-0}	0 hrs
G 11338	Chick 1-2 days old	2.3	16 hrs
P 9605	Rhesus monkey	3.33	1 day
G 11338	Chick 1-2 days old	3.6	16 hrs
G 11338	Chick 1-2 days old	4.6	1 day *
P 9605	Rhesus monkey	5.0	1 day
05	Rhesus monkey	6.5	2 days
505	Rhesus monkey	6.6	2 days

* No mosquitoes available for testing after 1 day.

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